ROYAL SOCIETY.

REPORTS TO THE MALARIA COMMITTEE,

1899–1900.

CONTENTS.

LONDON:
HARRISON AND SONS, ST. MARTIN'S LANE,
PRINTERS IN ORDINARY TO HER MAJESTY.

1900.

Price Two Shillings and Sixpence.

July 6, 1900.

STACK

WC
750
ROY

UNIVERSITY OF LEEDS
MEDICAL LIBRARY.

The University Library
Leeds

ET AVGE-BITVR SCI-ENT-IA

Medical and Dental
Library

Stack
WX 750
BOT

REPORTS TO THE MALARIA COMMITTEE

OF

LONDON

THE ROYAL SOCIETY.

CONTENTS.

30106 004182829

LONDON:

HARRISON AND SONS, ST. MARTIN'S LANE,

PRINTERS IN ORDINARY TO HER MAJESTY.

1900.

UNIVERSITY OF LEEDS
MEDICAL LIBRARY.

603568

REPORTS TO THE MALARIA COMMITTEE OF THE ROYAL SOCIETY.

"On Transmission of Proteosoma to Birds by the Mosquito: a Report to the Malaria Committee of the Royal Society." By Dr. C. W. DANIELS. Communicated by Dr. M. FOSTER, Sec.R.S., by direction of the Malaria Committee. Received February 13,—Read March 16, 1899.

I have the honour to report the results of my observations since my arrival here (Calcutta) on December 21, 1898.

2. Major Ronald Ross, I.M.S., after demonstrating and explaining to me his method of dissecting the mosquito, showed me in prepared specimens the pigmented bodies met with in the stomach walls of mosquitoes fed on birds infected with Proteosoma, and also the changes which these bodies undergo day by day. Finally he demonstrated to me the "germinal threads" in cysts in the stomach wall, in the fluids of the body, and in the cells of the veneno-salivary glands.

3. On my arrival there were in the laboratory, in test-tubes, several series of mosquitoes which had fed on birds infected with Proteosoma on the nights of November 30, December 10, December 12, December 15, and December 20.

Of each of these series Major Ross dissected specimens, and demonstrated in them the same bodies that he had already shown me in prepared specimens. He pointed out that in the older mosquitoes it was possible to predict from an examination of the fluid obtained on cutting the thorax the nature of the contents both of the "coccidia" (the term employed by Ross)* in the stomach, and of the cells of the veneno-salivary glands.

These points I readily observed.

4. Of the mosquitoes referred to I day by day examined those which died, and others which I killed. In these I was able to repeat the observations and, in insects belonging to the earlier series, to trace

* See note by the Malaria Committee appended to this Report.

the changes in the size and in the nature of the contents of the "coccidia."

I also examined a large number of mosquitoes caught about the laboratory, and others which had been raised from larvæ. In none of these did I find either "coccidia" in the stomach wall, germinal threads in the body fluids, or germinal threads in the cells in the salivary glands; nor did I find "black spores" (Ross).

5. Major Ross informed me that his published results were based on observations made in the hot season, when the temperature was 80° F., or over; and that now, as it was the cool season, I should find the changes progress more slowly, although the sequence of events was the same. My observations on the mosquitoes fed on December 20 and December 15 showed that this was the case. Major Ross also informed me that, with the lowered temperature, mosquitoes fed less readily, and that more difficulty was experienced in rearing them to a spore-bearing age.

These difficulties the use of the incubator was only partially successful in overcoming.

6. On the evening of January 1, following exactly in Major Ross's lines, I commenced a repetition of his main experiment:—

A large number of grey mosquitoes, reared from larvæ, were released in two mosquito nets.

In net No. 1 four birds were placed. On December 31 I had already found Proteosomata in large numbers in three of these birds, and in the fourth in moderate numbers.

In net No. 2 two birds, in whose blood no Proteosomata had been found, were placed. These two birds died two and three weeks later; on dissection no black pigment was found in their organs. Repeated examinations of their blood had failed to discover Proteosomata.

On January 2 none of the mosquitoes had fed, and on January 3 only two in net No. 1 and eight in net No. 2. On January 4, which was a warm night with a minimum temperature of 59·2° F., sixty-three mosquitoes were found in the morning gorged with blood in net No. 1, and were caught in separate test-tubes, which were then plugged with wool and placed in the incubator. Of the control series in net No. 2, where the non-infected birds had been placed, eighteen were caught and treated in the same way.

On the following two evenings, with minimum temperatures of 60·7° and 63·2°, sixty-three and forty-six mosquitoes were fed on the infected birds, net No. 1, and were kept for the preparation of specimens; and twelve mosquitoes were fed on the non-infected birds, net No. 2, bringing the number of the control series up to thirty-eight. At a later date eighteen mosquitoes were fed on a blue jay with numerous Halteridia.

On the third day the sixty-three mosquitoes, from net No. 1 (with

exception of those previously killed for examination or which had died), were released inside a clean net free from other mosquitoes. Birds free from Proteosoma were also placed in this net.*

In the morning all mosquitoes found inside were collected. Most of them had fed well. The minimum temperature during the night had been 63·2° F.

The mosquitoes were not fed on the following night as they were full of blood, which most of them voided during the night. Many died next day.

The remainder were given the opportunity of re-feeding every night after this; but as a spell of cold weather set in with minimum temperature of 44—49° F. (only on one night did it exceed 50° F.) few fed well or at all, and there was a consequent continued heavy mortality. Only one insect, which subsequently escaped in the night, being alive on the 10th day.

This method of feeding was very unsatisfactory in exceptionally cold weather. During the day the mosquitoes being kept warm in the incubator rapidly digested their food, whilst at night the cold rendered them torpid and they did not feed.

The control mosquitoes, of net No, 2, were treated in exactly the same manner, being fed on birds free from Proteosoma. The last died on the 13th day.

7. The results of the two series are as follows:—

Of sixty-three mosquitoes fed on proteosomal birds, forty-nine were examined, three were reserved for sections, one was too much decomposed for satisfactory examination; ten were not accounted for, having been lost in the nets.

Of the forty-nine examined two were killed on the first day—that is, under twenty-four hours, and possibly under twelve hours, after they had fed; no coccidia were found in these. Two more were examined the following morning—that is, under thirty-six and possibly under twenty-four hours after they had fed; no coccidia were found in these.

In two examined about 4 p.m. of the same day—that is, under forty-six and possibly not more than thirty-four hours after they had fed on the infected birds, minute pigmented coccidia were found.

The remainder were examined on the following days. The largest

* This is the method Ross employs to re-feed mosquitoes. If infected birds are employed to re-feed the insects, a younger generation of coccidia is produced; I therefore used sterile birds for this purpose.

The method works fairly well in warm weather, but there is always some loss, as the full number is never collected again in the morning. As the process is repeated over and over again, this loss becomes serious, the more so the longer the period required for maturation of the coccidia. Moreover, in a frequently repeated process of this kind there is always the possibility of an outside mosquito getting inside the net, and to that extent vitiating the experiment.

numbers (eighteen) were examined on the fourth and (twelve) on the seventh days, as on these two days the mortality amounted to this.

In all these mosquitoes, with one exception, coccidia were found—usually in numbers; in one there was only one coccidium.

The exception occurred on the ninth day; but as by that time the insects had been re-fed several times, the mosquito in question may have been an outside one which had effected an entrance.

Of forty-five mosquitoes fed on the infected birds and examined, more than thirty-four hours after, forty-four contained coccidia.

This I may say is a more successful result than in the other series I have seen.

The other two series of mosquitoes were used by all of us for the preparation of specimens, and no record was kept of the number of non-infected insects. Judging from my own examination, only about three-quarters of them developed coccidia. Their treatment had been somewhat different, as for several days half of them were not incubated.

Of the controls fed on birds free from Proteosoma, thirty-eight in number, and treated in the same manner, twenty-nine were examined and nine are unaccounted for—lost in the nets. None of the twenty-nine were examined on the first day, but one was on the afternoon of the second day. The largest number, seven and five, were examined on what would correspond to the fourth and seventh days, four were examined on the fifth and four on the sixth days.* In none of these twenty-nine were coccidia found.

Of the eighteen fed on the blue jay with Halteridia, twelve were examined from two to six days after feeding; none contained coccidia.

8. The coccidia (pigmented bodies) found on the second day measured 6—7 μ, some of them a little more. They were oval bodies containing scattered granules of black pigment, and had a sharp, clear outline.

I incised the stomach of infected mosquitoes and by repeated washing and compression with a cover glass was able not only to wash out the contents, but even to express the loosely attached epithelium, so as to leave the stomach a transparent clear bag. The majority of coccidia remained fixed to the outer wall, though in one of the mosquitoes I observed a few coccidia escape with the epithelium. On subsequent attempts to detach the coccidia by this process I failed to do so, though some coccidia would be ruptured.

The next morning the smallest coccidia measured 10 μ; some were

* It will be observed that these control mosquitoes were not, as the other series, collected on one, but on three nights. A very slight difference in breeze and light seems to affect the numbers that feed; any extra restlessness on the part of the birds has the same result.

12 μ. On the sixth day they were met with up to 30 μ; by this time the pigment had absolutely as well as relatively diminished.

In another three days some of them reached 60 μ; and in the last of the series examined (tenth day), there were coccidia measuring 70 μ.

The coccidia could now be seen to project from the outer wall of the stomach; very few contained pigment, and that only in small amount.

Some of the coccidia were clear, and others had a granular appearance; but in none were either black spores or germinal threads to be seen.

9. For the observation of the further development of the coccidium the early deaths of the mosquitoes, owing to the inclemency of the weather, rendered this series useless.

One of the insects infected on the night of January 5, and another infected on January 7, did reach this more advanced stage; and in the last of those fed on January 5, and which died on January 22, ruptured cysts, as well as numerous cysts containing mature germinal threads were found by me in the stomach wall; these threads were also found in the body fluids and in cells in the salivary glands. In one of the mosquitoes infected on January 5, which died on January 19, the coccidia had an appearance of striation.

In consequence of the effects of the unfavourable climatic conditions on the experimental insects, my observations on the development of the proteosomal coccidium were mainly made on mosquitoes infected November 30 and subsequent dates before my arrival, and on some infected on December 22.

On adding salt solution (15 grs. to the ounce) to an ordinary slide containing an infected mosquito stomach, and pressing on the cover glass, a projecting coccidium was ruptured; the contents poured out into the fluid, leaving the cyst wall still attached to the stomach.

The contents were seen to consist of a mass of shrivelled threads. This appearance I frequently observed in the other series of infected insects already mentioned.

These threads, Ross's germinal threads, are sickle-shaped bodies, about 14 or 15 μ in length. They stain with logwood or methyl blue, but not strongly. On adding water or Farrant's solution they lose their shrivelled appearance, and become more rounded. Nearer one end than the other is an unstained portion (? nucleus). They show no signs of movement; but as they are invisible in water, and only become visible when shrivelled by the salt or stained, it may be doubted if they have been seen alive.

If the thorax of the mosquito at a somewhat more advanced stage in the development of the proteosomal coccidium is incised, similar threads will be found in the fluid exuded, if salt solution is added. In this case ruptured cysts can be found in the stomach wall.

The relation of the infection to the veneno-salivary gland involves a difficulty not met with in any other part of the examination.

The dissection of the stomach is easy; that of the salivary gland in its entirety is not, and for some reason appears to be more difficult in the old infected mosquitoes. Any rough manipulation results in the detachment of the cells, and little more than the duct is left. In most cases, however, even in old infected mosquitoes, one entire gland, or portions of both, can be exposed in fair condition.

In every case where this was done, and in which germinal threads were found in the body-fluids, the germinal threads were also found in some of the cells of the salivary gland. I failed to find similar threads in the large number of salivary glands obtained from uninfected mosquitoes bred from larvæ, or caught about the laboratory, or from mosquitoes at the earlier stages of proteosomal infection.

The affected cells, as they have a granular appearance, can be distinguished with a low power; the unaffected cells are quite clear.

With a high power, if not very numerous, the isolated germinal threads can be clearly distinguished in the cells; they are recognised by their peculiar shape and shrivelled appearance (the examination must be made in salt solution). If numerous, the individual threads can be better made out in the cells of the salivary gland than in the coccidia of the stomach wall; but, as in the case of the latter, pressure on the cover glass will rupture the cell, and the germinal threads are then poured out.

The threads do not fill the cell. There is a faintly granular crescentic portion on the side most remote from the duct which, in many cases at least, is free from threads. The part of the cell in which the threads lie must be nearly fluid, as it permits oscillation of the threads to take place.

The whole of the veneno-salivary gland is never involved. In one dissection made by Ross the cells in both middle lobes and in no other part of the gland contained the threads. In several instances, where one gland has been exposed entire, the middle lobe alone has been involved; but in the majority all that can be stated with certainty is that the cells in one portion of the gland contain threads, and that those in the other portions do not.

On these points I have satisfied myself by repeated examination, though the appearances are by no means difficult to make out.

I have gone at some length into the description of this matter, as, so far, we have found no satisfactory method of making permanent preparations. All the preservatives at our disposal, with the exception to some extent of weak formalin solution, wrinkle up the delicate cells; and I have no confidence in this agent as a means of making permanent specimens.

The following specific observations made by myself on mosquitoes

dissected by Major Ross, Dr. Rivenberg, of the American Mission, who is working with Dr. Ross, and myself may be of interest:—

(*a*) Coccidial cysts full of apparently mature germinal threads; no ruptured cysts; no germinal threads in the body-fluids or salivary glands. Two observations.

(*b*) Cysts full of germinal threads; other ruptured empty cysts; germinal threads in body-fluids; germinal threads in salivary glands. Over twenty observations.

(*c*) Empty cysts in stomach wall; germinal threads in body-fluids of thorax; germinal threads in salivary glands; no cysts still containing germinal threads. Two observations.

(*d*) Empty cysts only in stomach wall; no germinal threads in body cavity; no germinal threads in well exposed salivary glands. One observation; the mosquito had been infected four weeks before death.

These observations fully confirm Ross's statement in every point. They indicate that the threads are formed in the coccidia; and that the germinal threads escape into the body cavity on the rupture of the coccidia, to be again collected in the salivary glands.

I should have liked to extend the series, but the continued cold weather renders it improbable that I shall be able to do so before I leave.

10. The infection of birds free from Proteosoma by the bites of mosquitoes.

On December 20, the day before my arrival, twenty-two birds were examined and found free from Proteosoma. On that night some of these birds were used for feeding the mosquitoes which had been infected on November 30 (?) and on the 24th and subsequent days; the remainder of the birds were used for feeding the mosquitoes first infected on November 30 and December 10, 12, and 15. In other mosquitoes of this series germinal threads were found in the salivary glands; and those which fed, when examined later, gave the results indicated in paragraph 9.

On December 30 Dr. Rivenberg and myself examined these birds; three of them had Proteosoma, two in large numbers.

On January 4 I examined them all except one which died on January 2; in this bird the heart's blood contained no Proteosomata, and the organs were free from pigment.

Five more of them had now Proteosoma; in every instance the parasites were very numerous. On January 6 and 7 I again examined them; three more had Proteosoma, also in large numbers.

On January 9 no more cases had developed; but on January 18 one of the birds had numerous Proteosomata. It was also ascertained that many of these birds which previously had been found to be infected had now recovered, whilst others showed but a few Proteosomata.

Thus twelve out of twenty-two birds (54 per cent.) became infected. This compares unfavourably with Ross's earlier results, as, in his published series, twenty-two out of twenty-eight (79 per cent.) were infected. But it is to be remembered that at the time this result was obtained the germinal threads were found at the end of a week; whilst in December the development was much slower, and took at least twice the time. It is much easier to keep mosquitoes alive during the first week after feeding them than it is to keep them alive for any subsequent period; moreover, in hot weather, such as Ross had worked in, mosquitoes bite more readily.

These results appear less unfavourable, if they are considered in connection with observations on the normal proportion of wild, uncaged birds, infected with Proteosoma at this season. Thus, earlier in the year, Ross, out of 111 wild birds, found Proteosoma in fifteen, or 13·5 per cent.; whilst I found at this season only one out of thirty, or 3·3 per cent., affected with Proteosoma.

It is possible that in the cold season the birds have a greater power of resistance; the validity of this conjecture is rendered more probable by the short duration of the proteosomal attack in my infected birds. Of the twelve, five died within the first week. In three of the survivors, in which the Proteosomata had been very numerous, no parasites could be found ten days after the commencement of the invasion; in one in which they were never numerous none could be found on the fifth day. In the other three very few are now found, though at first they were numerous.

The recovery of these birds and the death of the mosquitoes fed on them diminishes the chances of much future work on this line during the time remaining to me here.

11. Mention has been made of the differentiation of the coccidia (previous to the formation of the germinal threads), according to the appearance of their contents, into clear and granular; the evolution of the latter into the coccidia containing germinal threads can be traced day by day. This differentiation was clearly visible in my series.

In a minority of the coccidia, and in most infected mosquitoes, when the germinal threads are mature, certain black tubular bodies are to be found in cysts with otherwise clear contents. These black tubular bodies were frequently met with in the series of mosquitoes infected in November and December. Most of these mosquitoes contained some coccidia with black tubular spore-like bodies; though in a few insects all the cysts contained germinal threads only. In some cysts the black spores were numerous, and occupied the entire cyst; in other cysts there were only a few. In most instances germinal threads were not found in the black spore-bearing cysts; but there were a few such cysts in which it was doubtful whether germinal

threads were present or not, or whether the appearance arose from overlying threads which had escaped from a neighbouring capsule.

These black spores are very resistant; I have seen some which had been kept in water for months by Ross, and which had undergone no visible change. They withstand irrigation with liquor potassæ.

When the cysts are ruptured the black spores are to be found all over the body of the mosquito, but not included in cells. They do not seem to accumulate in any particular organ.

The most plausible view of the nature of these black spores seems to be that held by Major Ross, viz., that they are "resting spores," and that through them, by another cycle, the Proteosoma can be propagated in conditions unfavourable for direct propagation by mosquito-insertion into a warm-blooded animal.

If this be the case, three courses suggest themselves :—

(*a*) From the black spores may arise bodies capable of non-parasitic life (and possibly of reproduction), which at certain stages of their existence, and in certain conditions, on introduction into a warm-blooded host by inhalation, through drinking water, or even by injection by a mosquito or other blood-sucker in transferring them from the medium in which they live, may resume parasitic habits.

(*b*) That they may be ingested by mosquito larvæ, and in them undergo such development as will result in the formation of germinal threads in the adult mosquito, which, in turn, may be injected into the appropriate bird.

(*c*) That they may, if swallowed or inhaled by an appropriate warm-blooded host, so develop as to reach the circulation and pass into the sporulating phase.

Such experiments as have been made on this subject are inconclusive; and it is obvious that until the nature of these "black spores" is determined we cannot exclude, even for Proteosoma of sparrows, the possibility of any one of the many possible alternative channels of infection. Intervention of the mosquito intermediate host may be only an occasional requirement.

Still less are we justified in concluding that malaria in man can only be acquired through and directly from the mosquito; or in devoting our attention exclusively to that channel.

12. I have made myself familiar with the Proteosoma in sparrows, and the Halteridium in pigeons and crows.

In one specimen of a "blue jay," also, I found a very abundant Halteridium infection; the parasites in this instance had some peculiarities which I hope to work out if we can procure more of these birds. The bird I had died before I had completed my observation; I have preserved the organs as well as specimens of the blood in the heart.

13. In the cardiac blood of this jay there were numerous filariæ. They were sheathless, sharp tailed and fairly active, and had locomotory movement. They were of two sizes; in the shorter the tapering of the tail was much more abrupt than in the longer. Neither showed any extension or contraction.

Adults of one species only, three females and five males, were found in the subcuticular connective tissue, and in that round the trachea.

They were much longer and thicker than *Filariæ clava* (Wedl) or than the filaria described by Mazzini in the pigeon.

The females have the usual double ovary terminating in a vagina which appears tubular near the vulva situated near the caudal end of the body. The mouth is terminal and unarmed; the anus is subterminal.

The male has two spicules of equal length. The thickness of these worms, and the fact that when placed in weak formalin (2 per cent.) the cuticle burst in its entire length, will make them suitable for determining some of the disputed points in the anatomy of the Filaridæ.*

14. The difficulties in connection with human malaria are increased by the present plague scare. The suspicion of the natives about inoculation, makes them averse to any intercourse with European medical men.

By rewards, however, we have been able to get two fair cases of tertian fever, and three cases with crescent plasmodia—two of them with crescents in considerable numbers. On these cases we have fed mosquitoes—the common grey, and two varieties of "dapple wings" (large and small) in most points closely resembling those in which Ross had previously found pigmented cells after feeding on a patient with crescents. So far our results have been negative; but, in view of the peculiar climatic conditions, and of the possibility of the first stage, that of formation of coccidia, being inhibited by the cold, we are not prepared to accept these results as conclusive.

15. With Major Ross, I have examined the organs of some persons (eight) who died of kala azar. This appears to be an infectious disease, indistinguishable at first from malaria. Chronic in character, it continues for months and becomes associated with enlargement of the spleen and liver, and progressive anæmia. The present opinion of most of those who have been deputed to investigate kala azar, as well as of those with longest and most intimate experience of the disease, is strongly in favour of the view that it is malarial in origin.

The melanin or black pigment was absent in the organs of some of

* Judging from the description of the embryos, it is probable that these blood-worms of the Indian blue jay are identical with those found by Manson in Amoy, China, in the magpie (*Pica media*) and the gray mina (*Gracupica nigricollis*), in which case the mature form of one will be found to lie in the pockets of the aortic and pulmonary semi-lunar valves (*vide* 'Journ. of the Queckett Micro. Club,' vol. 6, p. 130, No. 44, August, 1880).

the cases I examined; but in all but one yellow pigment was present in the liver, and in most in the kidneys and spleen also, indicating hæmolysis. The iron reaction with acidified potassium ferrocyanide was obtained in the spleen in three instances and, in one, in the liver also.

So abundant and chronic a hæmolysis in cases of malaria, continued moreover after the parasite has ceased to be present (at any rate in sufficient numbers to be found in the peripheral blood or to cause appreciable deposit of melanin in the organs), raises the important question as to the possibility of the differentiation of parasites, with imperceptible morphological differences, by their toxic or hæmolytic properties.

16. Hæmoglobinuric fever seems to have been fairly common of late in some parts of India. I am collecting information, and have requested the editor of the 'Indian Medical Gazette' to insert in that Journal a series of questions on the subject. Hæmoglobinuria does not occur in kala azar, notwithstanding the great amount of hæmolysis which takes place in that disease.

I regret the length of this report, but the main subject of it, Major Ross's researches, cannot be dealt with in a few words, as they supply a basis for our future operations.

[It is necessary to point out that the word "coccidium" has been used by Major Ross and in Dr. Daniel's report above printed in a peculiar and not readily intelligible sense. "Coccidium" is the name of a genus of Sporozoa established by Leuckart in 1879 for the cell-parasite of the rabbit's liver, called *Coccidium oviforme*, and other allied species. "Proteosoma" is the name given by Labbé to another genus of Sporozoa parasitic in the blood-cells of birds. When Major Ross states in his report, dated May 21, 1898, that certain "parasites are a development in the mosquito of Proteosoma in birds; and to judge from their structure and mode of growth so far as yet observed, I take them to be *coccidia*," he is using the generic term "coccidium," to describe some phase in the growth of the species of a distinct genus, Proteosoma.

Apparently, what Major Ross intends to indicate by the term "coccidium" is an ovoid firmly walled corpuscle which increases in volume from about 1/2000th inch in length to four or five times that size, and then breaks up into a mass of filiform spores radiating from a central granular mass.

In this mode of spore formation these bodies have resemblances to the true coccidia, which present themselves not only as oviform corpuscles but as cysts with sickle-shaped or filamentous spores. It is, however, not legitimate to apply the generic term "coccidium" to a phase of growth of another genus.—LISTER, *Chairman of the Malaria Committee.*]

"The Malarial and Blackwater Fevers of British Central Africa." Being a Report to the Malaria Committee of the Royal Society. By J. W. W. Stephens, M.D., and S. R. Christophers, M.B. Received December 10, 1899.

The parasite found in these fevers belongs to the æstivo-autumnal type, and so far we have not seen in any case the simple tertian or quartan parasites.

In the examination of fresh blood films the parasite is seen either:—

(1) As an unpigmented body which, from its earliest to its latest phase in the peripheral circulation, presents no trace of pigment. We have never observed the brown shimmer (einen braünlichen Schimmer) mentioned by Koch in his description of the parasite found by him in cases of malaria in German East Africa. Or

(2) As a body which in quite early stages presents one or two or more pigment grains, easily discernible; and these may show motility.

For staining purposes we employed either—

(1) A solution of gentian violet, freshly prepared by adding a few drops of the saturated alcoholic solution to a watch-glassful of water. This solution stains the parasites and the leucocytes very deeply, in half a minute or less; and frequently leaves the red cells almost completely unstained, or only a faint yellowish-brown, and does not form precipitates. The films are previously fixed in a mixture of equal parts of absolute alcohol and ether.

Or (2) With a solution of hæmatin (a modification of Thin's formula)—

Hæmatin	2 grammes.
Alcohol (90 per cent.) ...	50 c.c.
Alum	50 grammes.
H_2O	1000 c.c.

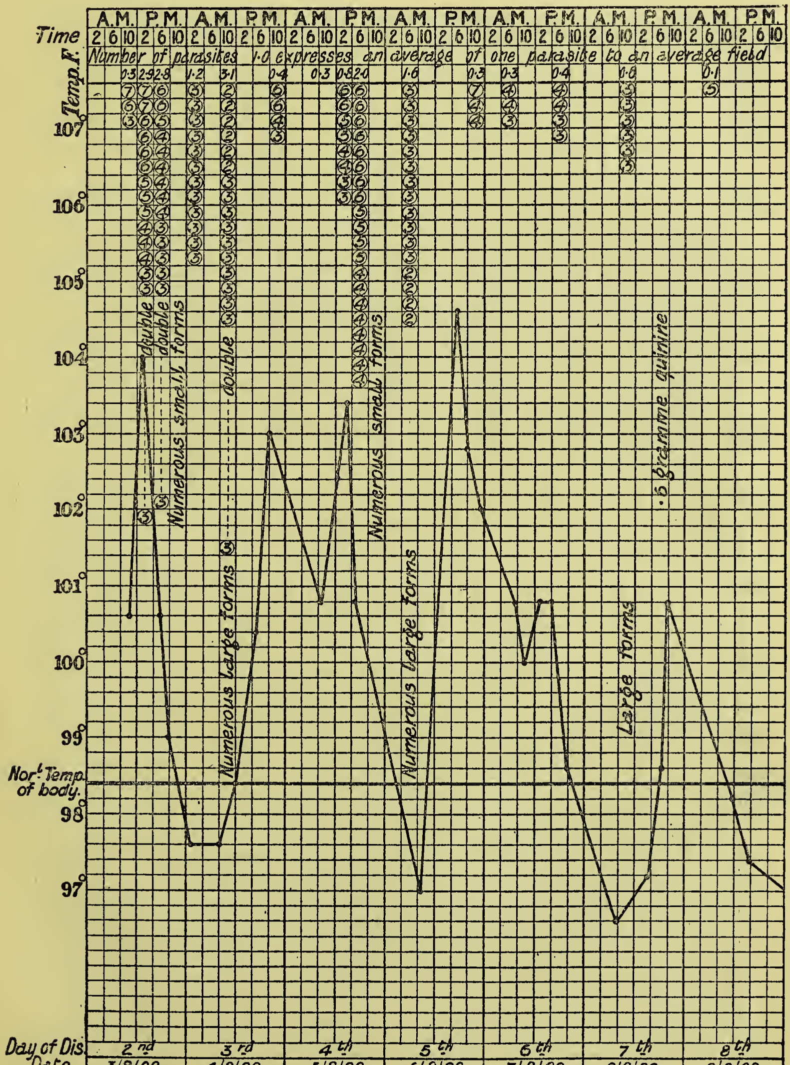
This solution gains in rapidity of staining power by keeping, and is very active when it has deposited a fine precipitate on the sides of the bottle. We have not (as practised by Thin) subsequently washed in alum solution.

Films after previous fixing are left in this solution for five minutes or longer, according to the age of the solution. The necessary time has been reached when the film has a faint brown hue. If now on examination the nuclei of the leucocytes are well stained, it will be found that the parasites also show clearly.

The nuclear networks are stained with a beautiful sharpness and clearness, while the cell body is untouched; so that, for instance, in the large mononuclear leucocytes pigment grains in the cell body can

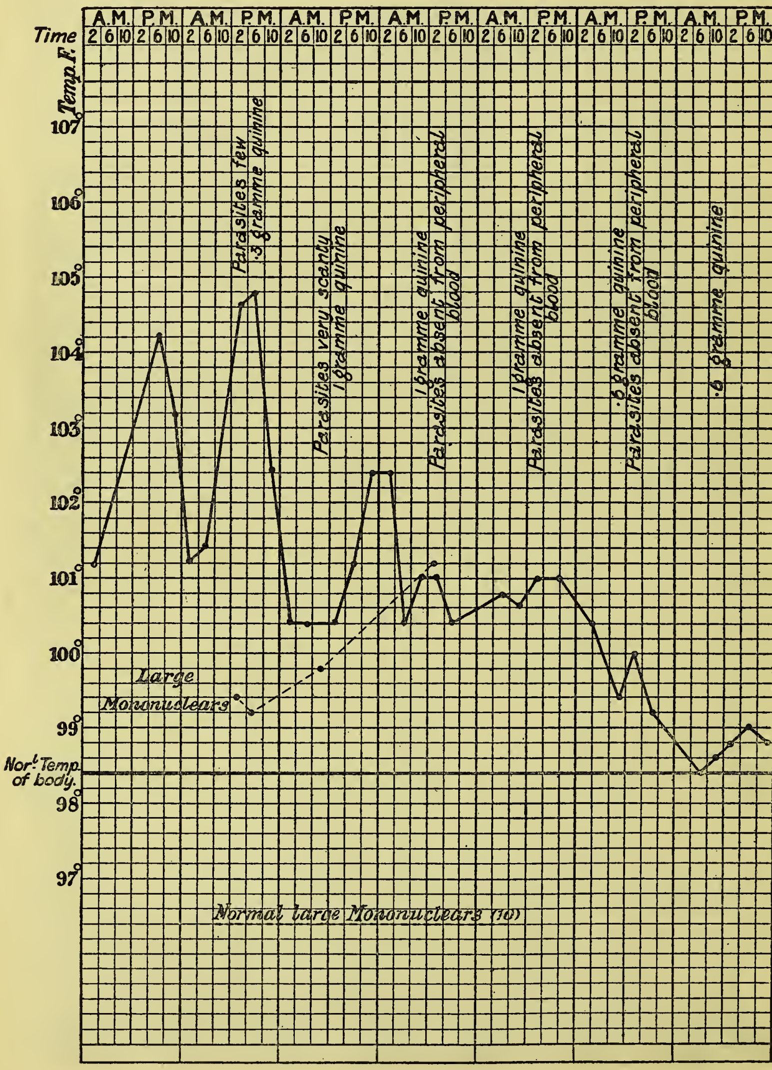
be seen with great distinctness. The red cells, if the specimen has not been overstained, take on a faint greyish-brown colour, against which the parasites stand clearly out. The different parts of the parasite, moreover, stain in different grades of intensity. This method being easy and rapid and giving delicate details of structure, we have made much use of in routine examinations. It further has the great advantage that it does not stain vacuoles as methylene blue does.

Chart I.



The parasite in the youngest form that we can distinguish appears as a minute ring of delicately staining matter, surrounding a space which even at this stage can occasionally be seen to be of a different refraction from that of the external red cell body. Its diameter is equal to one-tenth to one-eighth that of the red cell. The parasite when it has reached a diameter of one-sixth to one-fifth already shows further structures. Thus, in addition to the delicately stained ring

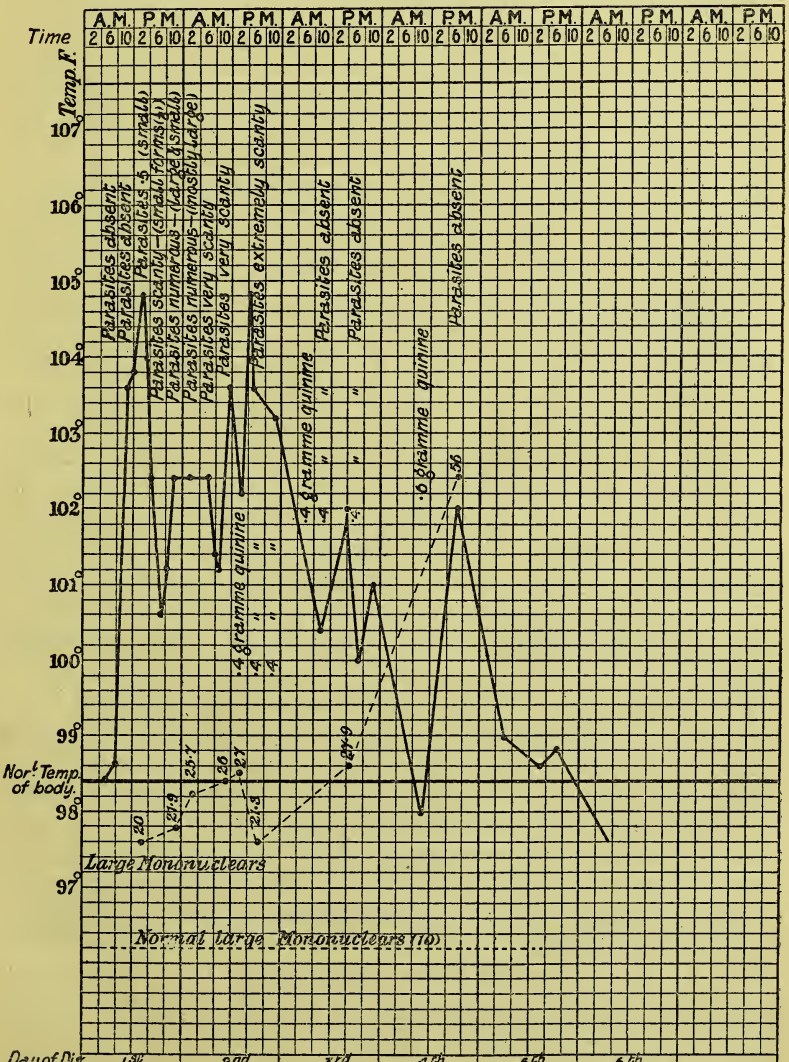
Chart II.



(chromatic substance), we find a deeply stained body (chromatic body) somewhere on the periphery of the ring; and also one or more minute particles (chromatic particles); these also stain deeply.

The central achromatic substance at this stage is sharply defined, by its white refraction, from the dull grey of the red cell. The chromatic body has the appearance of being applied to the side of the parasite; its shape is variable, it may appear as a small particle or rod, and

Chart III.



frequently it consists of two closely allied portions. Though most frequently at the margin of the parasite, yet it may be found in the centre of the achromatic area, free from any connection with the chromatic substance.

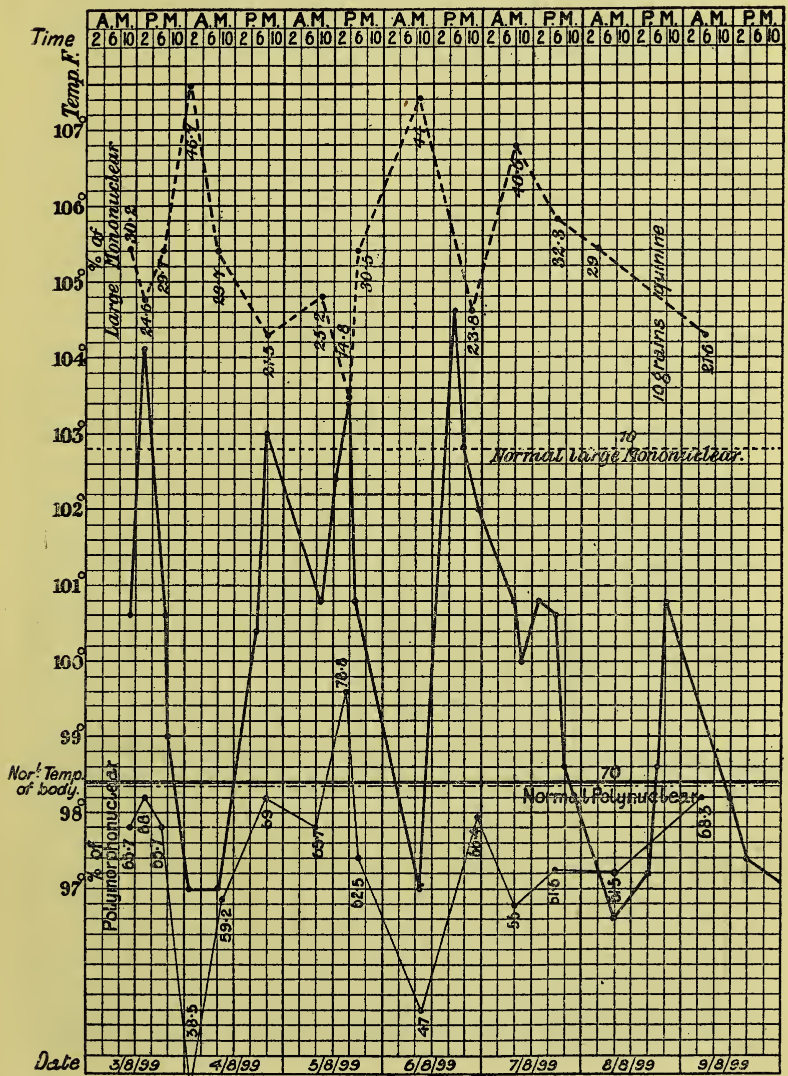
At this, or a later stage, it is seen that the parasite may be applied to the red cell (*accolé*). Parasites either clearly project beyond the rim of the corpuscle to the extent of one-fourth to one-third of their own diameter, or, what is more common, the parasite in optical section appears as a deeply stained body applied to the circumference of the red cell. At other times, in addition to the external portion, there is an internal feebly staining concave portion.

As the parasite increases in size, and is now one-fourth to one-third in diameter, we observe an increase in the amount of chromatic substance; and this mainly at one part of the circumference, giving the parasite an oval shape. In this chromatic portion we observe, now or even earlier, generally two, sometimes more, minute unstained areas. These very commonly occur in juxtaposition in the centre of this lateral expansion. It is mainly owing to this lateral expansion that the parasite further increases in size, until it has attained to one-half to two-thirds in diameter. The parasite then at this stage consists of a central achromatic area, a chromatic body, and a laterally developed chromatoplasm. It is in this much developed lateral area of chromatoplasm that we can observe further signs of differentiation of structure. This takes the form of a division of the chromatoplasm into two or more triangular or crescentic bodies. In the centre of these are situated the unstained areas mentioned above. When two of these bodies occur they are not uncommonly arranged symmetrically, with their bases applied to one another. The arrangement may, however, be irregular, aud again one body may stain more deeply than another. At other times this arrangement cannot be satisfactorily made out. Whether or no it possess any developmental significance we cannot at present assert.

The typical appearance of the parasite at this stage is an oval body. As the parasite reaches one-third in diameter the chromatic body is more frequently found encroaching on the achromatic area, and is now often central in position. The chromatic substance is accumulated at a pole. The parasite now presents itself as a long oval body one-half to two-thirds in diameter; and the accumulation of chromatoplasm has occurred to such an extent at one pole that around the achromatic area very scanty staining material is left. The parasite appears to undergo still further changes in the peripheral circulation. Thus we find that the chromatoplasm, which hitherto has stained comparatively faintly, now shows deeply staining areas in its outer portion, and the achromatic area may become so encroached upon as almost to disappear. The chromatic body may still persist, but in certain instances it appeared to be

merged in this large development of chromatic substance. Still further we find that this newly-formed chromatic substance may be divided into three or more portions.

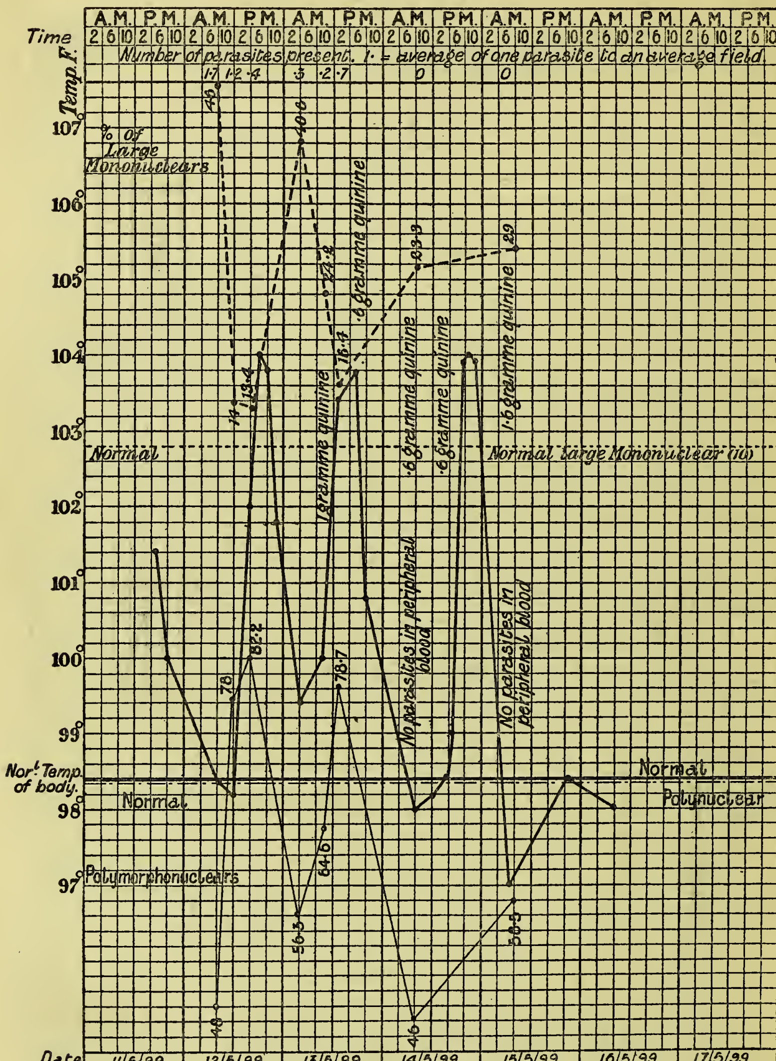
Chart IV.



The fully developed parasite, it has been noticed, often lies in a vacuole in the red cell. The corpuscles containing these developed parasites themselves show alteration in staining property. They

present minute clefts or spots scattered over the cell, staining slightly with hæmatin; they are most numerous in the immediate neighbour-

Chart V.



hood of the parasite itself. This appearance is, however, quite distinct from what is seen occasionally in red cells not containing parasites, but which show a cuneiform punctate appearance, probably constituting the so-called basophil degeneration of other authors.

In our experience the parasite does not, as a rule, develop pigment, even in its latest stage, but in those cases where we have seen pigment in quite young forms, we have been unable to distinguish any other morphological points of difference. Whether this implies the existence of two distinct parasites, we cannot as yet determine, for, in fact, in certain cases, where on one day the majority of the parasites appeared pigmented, on the following day the majority were unpigmented.

I. *Malarial Fevers.*

We may now consider the characteristics of these fevers under the following headings :—

1. Character of the temperature curve.
2. Relation of the parasite thereto.
3. Variations of the leucocytic elements.
4. Pigmented leucocytes.
5. The action of quinine.

1. *Character of the Temperature Curve.*—The uniformity of the temperature curves is sufficient to allow one to deduce a curve typical of the malarial fevers generally encountered.

In a case uncomplicated by quinine, existing for some days, the tertian character is well seen in the fall to normal, or generally below normal every third day; while on the alternate days the fall is approximately to 100° F. only (*vide* Chart I). We have thus between the two subnormal portions a curve which is characterised by an extremely rapid rise followed by a depression, then a rise, and finally a critical fall. The portion of the curve between the subnormal loops we may consequently regard as characteristic of a single febrile attack. In fact, the curve differs in no essential point from that described by Marchiafava and Bignami for malignant tertian fevers (*vide* Chart I).

This curve can be frequently detected even in cases complicated by quinine.

In other cases, especially perhaps in first attacks, the curves are most irregular, and we have been unable at present to ascribe to them any definite course.

2. *Relation of the Parasite to the Temperature Curve.*—We find that the period taken for the parasite to develop from the smallest to the largest size found in the peripheral blood is about eighteen hours, while the remainder of the forty-eight hours is passed in the internal organs (*vide* Chart I).

Again, if we reckon the period between two appearances of small forms or of large forms respectively, in both cases it is one of forty-eight hours (approximately).

The estimation between two periods of small parasites is more easily made at that point of the curve where they are most numerous. At

the summit of the curve the young parasites may be scanty, yet in certain cases there is a large outburst at the commencement of the crisis; it is at this point that the estimation is most easily made.

In many cases parasites at different stages of development can be found at any given point in the curve, but even in these cases there is always a preponderance of parasites of a particular size at particular times.

The fact that parasites of different ages exist at any particular time has led to the view that the time of development of the parasite is a very variable one; from twenty-four hours or less to forty-eight hours or more, but we think this may equally well be explained by a constant time of development with an inconstant output from the internal organs of young forms, which would naturally give parasites of different sizes in the peripheral circulation at the same time.

The distribution of parasites, especially the occurrence of numerous large forms in the apyretic period, and comparatively few small forms during the pyrexia, is a marked feature.

3. *Variation in the Leucocytic Elements.*—We observed, as has been done by several authors, the change in the proportion of the respective leucocytes in malarial fever. We determined to examine more closely than, as far as we know, has been done, the exact character of these changes, especially as we had reason to believe this might aid us in the investigation of the nature of blackwater fever.

It soon became evident that the variations in the leucocytes followed closely the variations in the temperature curve. Here again the variations are most easily observed in regular curves of the tertian type to which we have above referred, though similar changes are indeed to be observed in the majority of charts.

As far as our observations on the total number of leucocytes have extended, the diminution (which is frequently below 2000 per c.mm.) is constant throughout the temperature curve, but the change in the relative proportion of the constituents shows regular fluctuations.

The change consists especially in an increase in the large mononuclear elements, accompanied by a decrease in the polynuclear and small mononuclear. This change is most marked during the periods of apyrexia, and particularly in the normal or subnormal periods of intermittence.

Immediately succeeding the fall of the temperature, the mononuclear leucocytes may increase to such an extent, as to equal or outnumber the polynuclear. From this point the increase falls away, until there occurs a sudden interruption due to changes coincident with the ensuing febrile attack.

Should, however, an ensuing rise not occur, but the temperature remain normal, then there is a gradual decrease in the mononuclear elements until the normal relation is again reached.

As the temperature rises, the relative proportion of the apyretic period undergoes a sudden change, the polynuclear elements now reaching their normal value. This normal proportion continues during the pyretic period with a slight disturbance during the precritical notch, but otherwise the normal relation is retained during the pyrexia until again there is a sudden large mononuclear increase after the crisis.

Examples of this cycle are the following (*vide* also Charts IV and V):—

4.8.99	1 A.M.	97·6° F.	Large mononuclear ...... Polynuclear	46·7 38·5
	8 A.M.	97·6	Large mononuclear ...... Polynuclear	29·7 59·2
	5 P.M.	99·4	Large mononuclear ...... Polynuclear	21·5 69·0
5.8.99	9 A.M.	100·8	Large mononuclear ...... Polynuclear	25·2 65·7
	3 P.M.	103·4	Large mononuclear ...... Polynuclear	14·8 78·8
	5 P.M.	100·8	Large mononuclear ...... Polynuclear	30·5 62·5
6.8.99	9 A.M.	97·0	Large mononuclear ...... Polynuclear	44·0 47·0

In some cases the mononuclear increase is apparent during the pyrexia, the polynuclear increase not being adequate to cause the return to normal. We thus may have a varying mononuclear increase throughout the course of the fever; but even here the mononuclear increase is greatest at the end of the febrile period (*vide* Chart VI).

In other cases we have a polynuclear increase beyond the normal during the pyrexial period.

In cases with continuous high temperature lasting several days, we may find no mononuclear increase until the temperature subsides (*vide* Chart III).

In one case where the regular cycle we have described was very apparent, and at a time when the relative proportions of mononuclear and polynuclear elements were 47 and 49 respectively, there occurred (coincident with the onset of pernicious symptoms, access of severe vomiting and collapse) a rapid disturbance of this relation, the proportion now becoming 18 to 80, and this proportion, notwithstanding the subnormal temperature, continued till death six days later (*vide* Chart VII).

What produced this exceptional course, and under what conditions this leucocytic variation may possibly be absent, we do not know.

In the regular curves it is noteworthy that the mononuclear preponderance is found together with the occurrence of the large forms

of the parasite, while the small forms are accompanied by a polynuclear increase.

We have reason to believe this mononuclear increase may, under certain conditions, continue some time after the subsidence of the fever. The significance of this, its possible relation to immunity or to continued infection, we shall further examine when suitable cases present themselves.

In a count of the leucocytes recorded in our previous report, we said that the eosinophil cells had the value of 14 per cent. This value is in excess of what we have found in numerous subsequent counts where the value is usually less than 1 per cent. But occasionally we have met with specimens in which the value was approximately the former.

Some of the cases have occurred at a time when crescents were present in the blood; but the exact relation of this high value to the malarial fever we must leave for future consideration.

4. *Pigmented Leucocytes.*—The occurrence of pigmented leucocytes we have already described. The most characteristic of these are the mononuclear leucocytes containing one to two or more clumps of pigment, and ten to thirty fine grains scattered through the cell body.

These appear to us to be of great diagnostic value; their appearance is absolutely characteristic, and they give a definite proof in the absence of parasites of a recent infection.

Thus in many cases where quinine had caused the complete disappearance of parasites, which we had previously observed, we had still evidence of malaria in the occurrence of these leucocytes.

Pigment also is found in the polynuclear cells in a smaller or greater amount, but we regard the mononuclear cell with much pigment as peculiarly characteristic.

Isolated dots of pigment also are found in the leucocytes, but we should hesitate to make a diagnosis simply on the presence of these. It is perhaps necessary to point out also how easily minute tags of the nuclear network apparently or really detached from the nucleus can be mistaken for pigment. In specimens stained with hæmatin these tags are an almost constant phenomenon.

The detection of pigmented leucocytes may require prolonged search. Their absence must not necessarily lead to a decision against malaria, for our knowledge as to the conditions which bring about their appearance in the circulation is still incomplete. Thus, in a fatal case of blackwater, we had observed numerous parasites and pigmented large mononuclear cells in the peripheral blood. Both these completely disappeared after the administration of quinine; but post mortem in the spleen there were found large numbers of mononuclear leucocytes containing much pigment associated with numerous parasites of a spherical and ovoid type.

5. *Action of Quinine.*—The rapidity and completeness with which even small (*e.g.*, 0·6 gramme) doses of quinine will cause the disappearance of parasites from the peripheral circulation is a most striking phenomenon. Again and again have we examined blood films in cases, presumably malarial, with negative result, and in most of these cases we have found that the patient has been taking quinine. Nor can the absence always be attributed to the cessation of the attack, for the clinical symptoms may not abate, and the pyrexia may continue for days. The case may even terminate fatally without the presence of a single parasite in the peripheral blood. Had such a case come under one's notice subsequently to the administration of quinine, and had one based one's diagnosis simply on the presence of parasites, the conclusion would have been reached that the case was not malarial.

The absence of parasites in the presence of quinine is no proof that the case is not one of malaria. We still, however, have two means of arriving at a diagnosis in cases where the absence is due to quinine.

(1) The presence of the characteristic much pigmented leucocytes (mononuclear).
(2) The leucocytic variation.

Examination for the presence of this diagnostic sign should be made by preference during the apyretic period. An examination made during the pyretic period, if the variation is absent, is inconclusive.

It is true that a similar mononuclear increase has been observed in typhoid fever, but there are other means by which typhoid fever can be diagnosed.

That this reaction is exceptionally absent we have already stated.

II. *Blackwater Fever.*

We do not now propose to discuss the many views that are held as to the nature of this fever. We shall draw attention only to the facts which the five cases we have so far seen we believe establish. We shall allude to these facts under the following categories.

I. *Existence of a Special Organism.*—In all five cases, frequent examinations of the blood failed to show any *special* parasite or organism. In two cases cultures were made from the blood on agar, in both they remained sterile. In one case cultures were made from the spleen and heart's blood; in both pure cultures of *Staphylococcus aureus* resulted. Cultures made from the bile of the same case remained sterile. In case II which we have already reported, no micro-organisms or *special* parasites were found in the organs post mortem.

II. *Relation to Malaria.*—In discussions on blackwater fever, considerable stress is laid on the presence or absence of parasites. It is urged by some that their absence is due in most cases to the fact that

quinine has been taken, but no further evidence has been adduced by such observers in support of their view, that such cases without parasites are malarial. We have shown above, however, that two methods of proof still exist in cases where parasites are absent owing to the administration of quinine, these are namely: (1) The presence of pigmented leucocytes; (2) The leucocytic variation.

In all our cases parasites were absent, and in all quinine had been taken. In one case we had not the opportunity of applying the further means of diagnosis to which we have drawn attention.

The remaining four we shall show are in direct relation to a malarial infection.

Thus in case III the hæmoglobinuria occurred during the course of a typical malignant tertian fever, in which very numerous parasites were found previous to the blackwater and none subsequently.

In cases II, IV, V, no "intracorpuscular" parasites were at any time seen. (In case IV crescents were seen but they were extremely rare). In all, however, both pigmented leucocytes and the leucocytic variation occurred, so that, in spite of the absence of parasites, we feel justified in classing these also as malarial.

In two cases the diagnosis made solely on these grounds received additional confirmation, in one case from an examination of the organs (Case II, Report I), in the other by an occurrence, seven days after leaving hospital, of a severe malarial fever with numerous parasites. We are of opinion that in this case the patient was not exposed to the possibility of fresh infection. Had we relied then simply on the presence of parasites as a proof of malaria, we should have been unable to assign such an origin to three of our cases; but by these subsidiary means of diagnosis we have been able to class them all as malarial.

III. *Relation to Quinine.**—In all our cases the patients had taken quinine previous to the onset of hæmoglobinuria.

The intervals between the taking of quinine and the hæmoglobinuria were as follows:—

Case I.—Two doses of 0·3 gramme five and two hours respectively.
Case II.—The interval could not be determined.
Case III.—Twenty-three hours.
Case IV.—Ten hours and in a recurrence six hours.
Case V.—Seven hours.

These cases, and especially Case IV, in which a relapse of blackwater

* In procuring histories from patients; whether or no they had taken quinine, when they had their last malarial attack, whether they had been bitten by mosquitoes, &c., we have experienced great difficulty in arriving at the truth. We have no hesitation in discarding as valueless all statements which have not been submitted to rigorous cross-examination. We have found that patients and others are only too ready to volunteer statements which on close inquiry are found to be absolutely baseless.

seemed very definitely to follow the administration of quinine, seemed to us to lend support to the view held by several authors who attribute the onset of blackwater to quinine. We therefore endeavoured to ascertain if experiments in vitro could produce similar phenomena, but they have yielded negative results.

1. Thus we endeavoured to show the presence of a hæmolytic toxin in the hæmoglobinous urine. On our own blood it had no such action, we regret that we did not try the experiment on the patient's blood.

Nor did the serum of the patient, Case IV, hæmolyse our own blood, nor although the urine contained hæmoglobin for twelve hours after the experiments were made, did the patient's serum show any hæmoglobin on separation from the blood clot.

2. We endeavoured also to ascertain if any change in the resistance of the red cells existed, but this was also negative. Thus the isotonic point of the patient's blood and our own was identical.

Observations were made subsequently to a first attack, previously to, during, and subsequently to a second attack. These results are not conclusive, however, against the view that the resistance is lowered, for the less resistant corpuscles may already have been destroyed.

3. We next determined the resistance of the corpuscles to quinine solutions.

Thus 1 gramme of quinine hydrochlorate was dissolved in 21·4 c.c. of 1 per cent. saline by heating. On cooling, crystals separated out. The remaining solution was then diluted with 1 per cent. salt, so that in a series each dilution contained half as much quinine as the preceding tube. To each series was then added (1) patient's blood, (2) normal blood.

Patient.	Original solution hæmolysed.	1st dilution hæmolysed.	2nd dilution clumped.	3rd dilution clumped.	4th dilution clumped.
Normal.	Hæmolysed.	Hæmolysed.	Negative.	Negative.	Negative.

The *hæmolytic* action of quinine in vitro is thus the same for the patient's blood as for normal blood.

The action of quinine in solution in serum was also tried on the patient's blood. At a time when presumably the administration of quinine might bring about hæmoglobinuria, one of us took 2·0 grammes of quinine, and when subjective symptoms were pronounced, blood was taken and the serum allowed to separate. To this serum the patient's blood was added, but the result was again negative.

Though we have been unable to show in vitro any alteration in

tonicity or increased susceptibility to quinine of the blood of a blackwater patient, yet in reference to the view that quinine does exercise such hæmolytic action, we may suppose that it is unnecessary to ascribe any such direct action to quinine itself, but that rather by its lethal effect on the parasites a sufficient quantity of toxins from their protoplasm is liberated to bring about the hæmoglobinuria.

These experiments we have only had the opportunity of applying in one case, and they will necessarily require repetition and modification before we can deduce conclusions from them.

Since our return to England we have had a opportunity of seeing Professor Koch's report on blackwater fever. He finds that in twenty-three out of forty-one cases there were no parasites present, and that in those cases where present the rule was to find few. This it seems to us is explained by the fact that in all cases quinine has previously been administered.

No statement is made as to the presence or absence of pigmented leucocytes in the blood, nor is it explained what the nature of the "fever" or malady was that induced the patient to take quinine.

Further in the post mortem records no examination of the brain appears to have been made.

To our five cases may be added two, the organs of which have been sent us by Dr. Todd of Umtali and Dr. Elmslie of Bardawe respectively, in both these the spleen showed large deposits of black pigment, the other organs we hope to examine shortly.

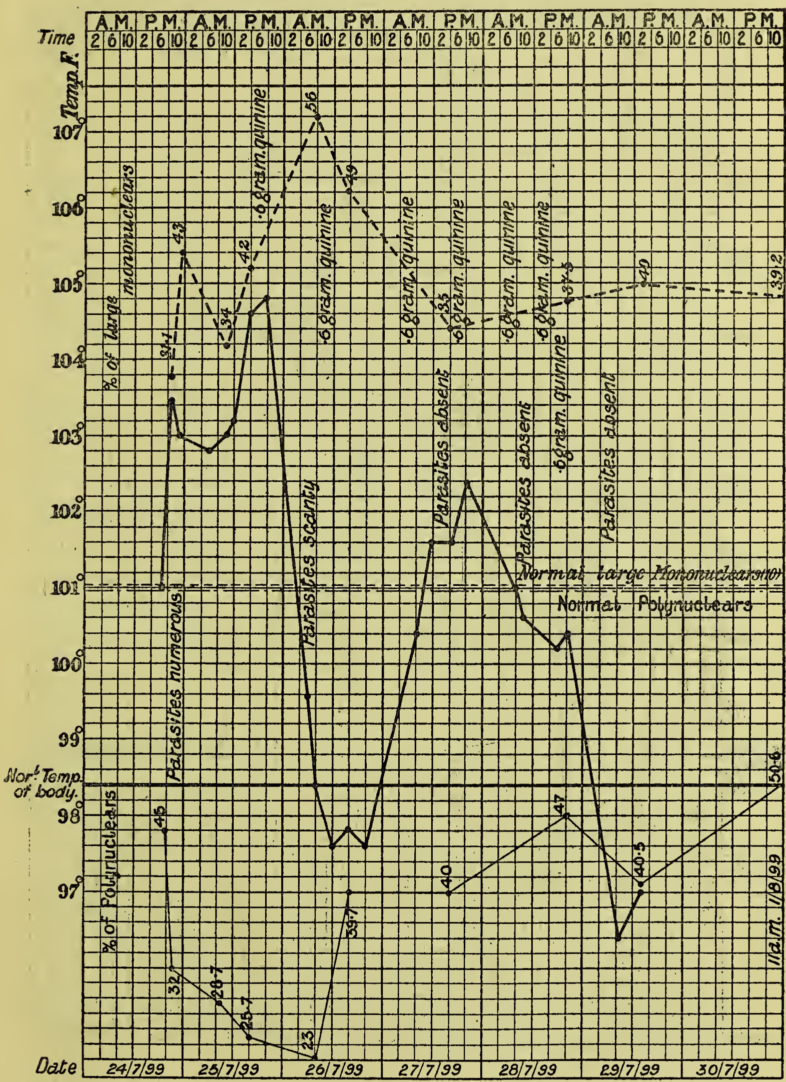
So that to sum up, we have seven cases of blackwater, all of which show a malarial infection, though of the five cases we saw during life only one showed parasites. It is necessary then to determine how many of these cases without parasites are really malarial.

Case I.—H., a plasterer, living thirteen miles from Blantyre, arrived in country February, 1896. Had febrile attack January, 1899. February 6th, 1899, 4 P.M. feeling cold and unwell, took 5 grains of quinine; 7 P.M. took 5 grains again. 9 P.M. passed an ordinary quantity of deep red urine; the specimen showed Hgb and red cells. 12 P.M. passed more, lighter. 5 A.M. February 7th more, still lighter. February 7th patient admitted to hospital. No Hgb was found in the urine, temperature normal and patient feeling quite well. No recurrence of symptoms. Numerous examinations of the patient's blood in the fresh condition were made and no parasites were found.

Case II.—J.R., age 28, engineer. Came to B.C.A. in August, 1897. Except for a few months spent at Chinde, he has been living at Inpimbe in a mud and wattle house on the bank of the Upper Shire river. He has not had fever previous to living in this country. Whilst at Inpimbe he has had occasional slight attacks of "fever."

Chart VI.

Case I.

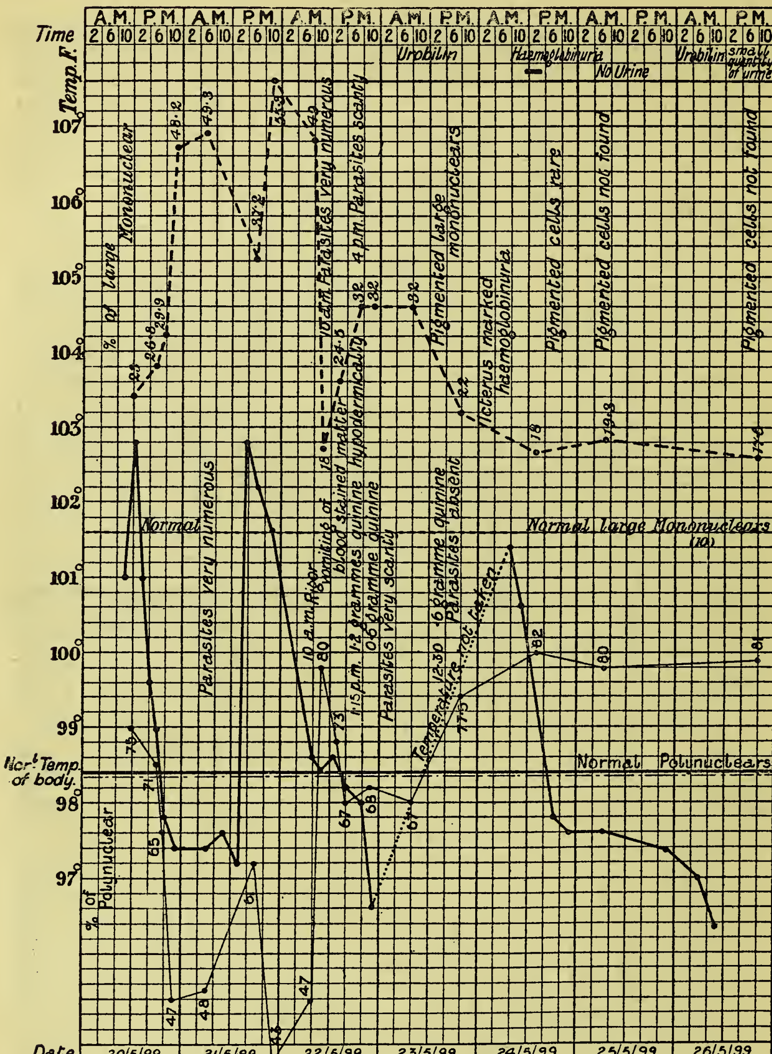


For a week previons to the attack of blackwater, he complained of being out of sorts. On the morning of 18th March he took 5 grains of quinine. At night his temperature was 102°. Next morning he took 10 grains of quinine. At 4 P.M. his temperature was 103°.

About 2 A.M. on the 20th he was suddenly seized with severe vomit-

Chart VII.

Case II (*vide* also Chart X).



ing. Three hours later he had two severe rigors at an interval of half an hour. Next morning his urine was black.

Dr. Grey, who arrived at midnight of the 20th, states that during the 21st and 22nd the most prominent signs were—

1. A temperature of above 100°, rising about midnight on the 20th and 21st to 104°.

2. The passage of an extremely small quantity of urine (2℥ since his arrival). The urine was of a dark mahogany colour.

3. A pronounced yellow coloration of the skin, first seen on the morning of the 21st, and afterwards fading so that shortly before death there was only a faint trace upon the legs.

4. Frequent vomiting. At first of bright green vomit, later of dark grumous material.

5. Marked hiccough occurring frequently throughout the illness.

6. An enlargement of the spleen, so that it could be felt three fingers' breadth below the costal margin.

Twelve hours before death the fresh blood showed no parasites. The red corpuscles were enormously reduced in number, being only 795,000 per c.mm. The hæmoglobin was reduced to 30 per cent. of normal. Leucocytes were 10,000 per c.mm.

Up to five or six hours before death patient was quite clear mentally, after that he became delirious and semi-conscious. Some hours before death his respirations which had been twenty-six per minute gradually increased to forty-eight per minute.

Death took place shortly after a convulsion at 5 A.M. on the 23rd. One hour before death the red corpuscles were 760,000 per c.mm.

A partial autopsy was made one hour after death.

The following were the chief features:—

Tissues were very pale—fat very yellow—blood extremely thin, dark and fluid.

Liver was slightly enlarged—smooth. It contained a large amount of blood which flowed freely on incision. Substance of liver was pale and rather waxy looking.

Spleen was enlarged, measuring 7 inches in long diameter—firm in consistence—no evidence of perisplenitis.

Kidneys were slightly enlarged—the capsule was adherent in patches. There was no marked congestion. On passing a knife over the cut surface a brownish fluid resembling the urine was expressed, but no visible blood. The cortex was dark brown and soft in consistence.

Stomach was contracted—there were no petechiæ.

Intestines appeared normal.

Bladder was empty and contracted.

Red marrow was normal in appearance.

The thorax and brain were not examined.

Examination of Blood-films, Urine, and Sections of Organs.

Blood.—The patient was first seen on the 21st March, 1899, early morning, twenty-four hours after onset of acute symptoms. Films were taken at four-hour periods (and sometimes more frequently) from this time till death on 23rd, 5 A.M. The red cells in these specimens

were normal in appearance. No nucleated red cells were seen. The leucocytosis was a marked feature, so also the large quantity of platelets.

Although in none of these specimens could we detect parasites, yet there was evidence of a recent malarial infection in the presence of pigmented leucocytes in the films taken on the 21st, 22nd, and 23rd respectively. The pigment was black, occurring in coarse spicules and also in finer granules, and, as far as we can judge, is identical with malarial pigment.

The leucocytic variation will be seen on reference to Chart X.

Urine.—Dark, brownish-red in colour, depositing a dark-brown, granular sediment on standing. Hgb = 20 per cent. of normal blood. No met. Hgb. The oxyhæmoglobin bands gave the reduced spectrum of Hgb on addition of Stokes's fluid. Boiled with soda, on cooling, the urine gave deep hæmachromogen bands, indicating, probably, the existence of reducing substances in the urine.

Much albumin. No bile pigment. Pavy's fluid showed reducing bodies.

The sediment (centrifugalised) consisted of a large quantity of brownish-yellow bodies 1—7 μ in diameter; also minute granules. They are also seen in the collecting tubules of the kidneys, and are probably identical with "granular cast" material. No red blood cells. There was much granular epithelium stained yellowish.

Torula, micrococci, and bacilli were also present.

Sections.—Sections of kidney, liver, spleen, and bone marrow have so far been examined.

Sections in paraffin were stained with methylene blue, gentian violet, and Bright's stain for micro-organisms; but, so far, no evidence has been obtained of their existence in the tissues (cultures from the organs in the fresh state were not made). The pathological changes shown in the tissues are as follows:—

Spleen.—Films of splenic pulp, stained in various ways, showed no evidence of the existence of malarial parasites or crescents. There were present, however, here also numerous pigmental leucocytes. Paraffin sections.—

The blood in the sinuses showed no parasites, but numerous bodies having the character of platelets. By Bright's stain long masses of fibrin were seen. The most characteristic appearance was the deposit of pigment. It occurred chiefly in the cells of the splenic reticulum. Secondly, in large cells with nuclei, staining feebly and diffusely (macrophages). The contents of the latter included also granular *débris* (eosinophil) and red cells. Thirdly, in large mononuclear cells. The pigment possesses a greenish-black colour, is often arranged in small, spherical clusters. The pigment granules are surrounded by a clear space. No coarse granules of pigment were seen. Yellow pig-

ment also occurs in small quantity in the macrophages, not exceeding the normal in amount. The dark pigment on the whole remains unchanged by HCl and K_4TeC_{76}. In the macrophages and, to some extent, elsewhere a blue reaction is obtained.

Very little pigmentation was seen in connection with the Malpighian bodies. The spleen was stained further, according to McCallum's method, with watery hæmatoxylin for inorganic iron salts; the result was negative.

There was no amyloid change here or in any other of the tissues examined.

Liver.—The evidences of pathological change were greater here than in the spleen.

The most noticeable changes were :—

(i) Small necrotic foci.

These were situated laterally to the intra-lobular vein, occasionally extending to the periphery of the lobule. The hepatic cells in these areas were much shrunken, and stained badly, and their nuclei were distorted, and stained diffusely.

There was no leucocytic infiltration in the neighbourhood of these areas.

(ii) Small thrombi.

These are found in considerable numbers in the sub-lobular veins. The thrombi show here and there in their substance small pigmented cells.

(iii) Pigment.

It is greatly in excess of that found in the spleen. It occurs in three forms :—

(1) Yellow pigment situated in the centre of the liver cells.
(2) As greenish-black pigment occurring chiefly in large swollen cells lying in the liver capillaries (endothelial); also occasionally in normal endothelial cells. The pigment is gathered into spherical clusters as in the spleen, is frequently surrounded by a clear area. In the swollen endothelial cells the pigment may occur in large spherical masses. Here it is found together with yellow pigment.
(3) In leucocytes in the small blood vessels.

In sections treated with HCl and K_4TeC_{76} the yellow pigment in the liver cells gives a uniform blue reaction; the reaction occurs also in association with the black pigment of the endothelial cells; but in this case there was always much black pigment unchanged. No inorganic iron salts were present (McCallum's test).

Kidneys.—(i) Glomerular changes were absent. Nor elsewhere were there any signs of acute inflammatory process.

(ii) The convoluted tubules showed much change. The cells were

in process of active disintegration, the lumen of the tubule being filled with a granular mass. The cells had lost their striation, were very granular, many of the nuclei also showed signs of degeneration.

(iii) The straight (and collecting) tubules were filled with shed epithelium and masses of granules. The large collecting tubules were also filled with granules resembling, but rather larger than, those seen in the urine.

(iv) Under the capsule were patches of young fibrous tissue with large multinucleated cells containing yellow pigment. No dark pigment was found in the kidneys.

Red Marrow.—There is much pigment in the marrow of the sternum. It occurs (1) in the capillary endothelium; the pigment-containing cells are swollen; (2) in large branched cells of the narrow stroma; (3) in pigment-laden cells (leucocytes) scattered throughout the marrow. In these latter cells yellow pigment is found together with the black, but in the other case the black pigment occurs alone. No parasites were found.

Case III.—Has been two years in B.C.A. The first year was spent at Likoma, Lake Nyassa. He had fever there twice, and also "jaundice." Most of the second year at Mponda's, near Fort Johnston, Lake Nyassa. Had fever there in March, 1898. He had fever at Fort Johnston just before leaving for Blantyre. He was in Blantyre a few days when he was attacked by severe pain in the muscles (of the chest). Two to three days afterwards the present fever developed, and the patient was first seen on 19.5.99.

Up to the morning of the 22nd the fever was of the ordinary tertian character, with numerous parasites.

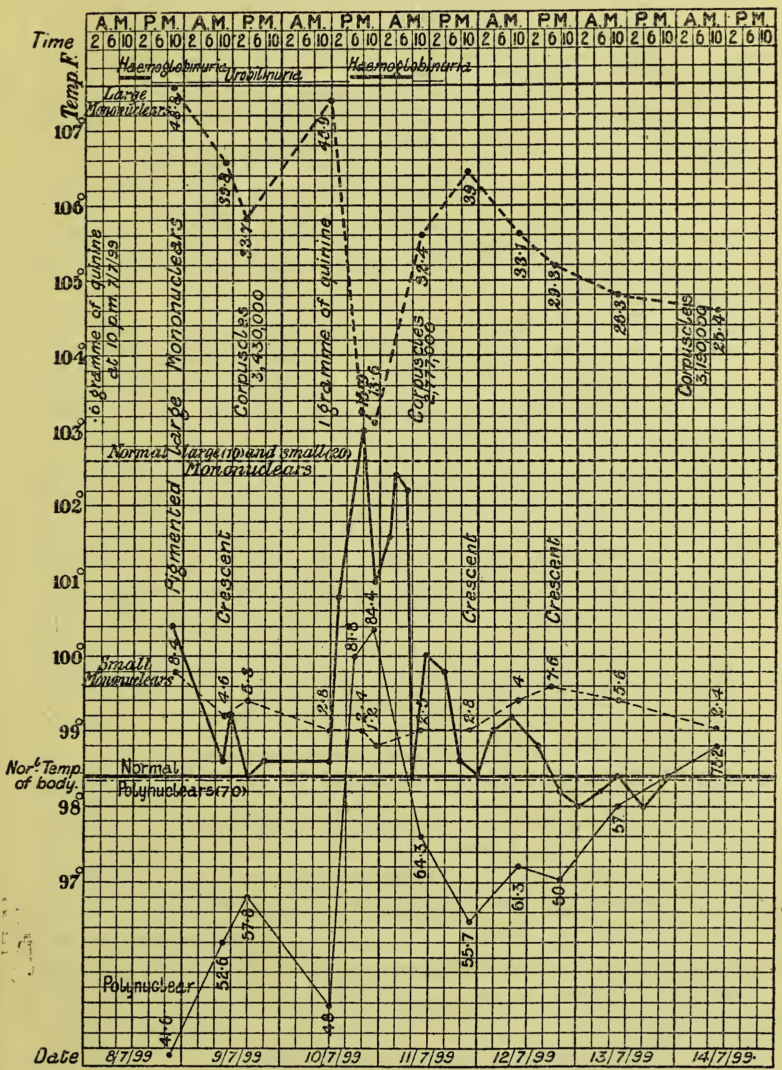
22.5.99., 10 A.M. Whilst the temperature was falling to normal he became much collapsed, had severe vomiting (the vomit being blood stained) and much shivering. At this time a sudden change took place in the relations of the leucocytes, the great mononuclear preponderance which was accompanying the fall in the temperature curve suddenly gave place to an increase of the polynuclears—a relation which lasted till death.

22.5.99. 1.15 P.M. Quinine was, for the first time, administered, 1·2 grammes being given hypodermically.
10 P.M. 0·6 gramme of quinine hypodermically.
23.5.99. Parasites were very scanty. A small quantity of urine passed in night contained no hæmoglobin.
12.30 P.M. 0·6 gramme quinine.
24.5.99. Distinct icterus.
11.30 A.M. Hæmoglobinuria. Parasites absent.

From the 25th to 28th patient became very weak. Icterus was very

Chart VIII.

Case III (*vide* also Chart VII).



marked on the 25th, and continued till death. There was also troublesome hiccough.

27th. Fæces contained blood.

28th. Double parotitis.

Death occurred on the 29th.

Hæmoglobinuria and urobilinuria.—

22.5.99. 7.30 A.M. Urine clear.
1.15 P.M. 1·2 grammes quinine hypodermically.
7 „ Urine clear.
10 „ 0·6 gramme quinine hypodermically.
12 midnight. Urine clear.

23.5.99. 12.30 P.M. Urine clear, 0·6 gramme quinine.
During night urine clear.

24.5.99. 11.15 A.M. 84 c.c. Dark urine. Hæmoglobin.
12.45 P.M. 28 c.c. Dark urine. Methæmoglobin granular casts and granular sediment. Attached to some of the casts were small masses of reddish crystals (? hæmatoidin).

25.5.99. No urine passed.

26.5.99. 7.45 P.M. Dirty brown urine. On centrifugalisation, pale yellow. No hæmoglobin. Albumin. Many casts in sediment, some cylindroids with adhering yellow pigment. No bile pigment.

27.5.99. 6 P.M. A test-tube full. Very dark urine, depositing a greenish-black sediment on standing. The sediment consisted of masses of spherical bodies varying in size from the diameter of a red cell to minute particles. They were stained a yellowish-green colour, and had the character of the granules of coarse tube casts. Adhering to the bladder epithelium there was much refractile greenish-yellow pigment. Hæmoglobin absent, much albumin, and no bile pigment.

Blood.—The destruction of corpuscles was not great.

24.5.99. 11.15 A.M. Hæmoglobinuria.

24.5.99. 4 P.M. Red corpuscles 4,000,000 per c.mm.

Leucocytes.—24.5.99. 4 P.M. 22,000 per c.mm.

Up to 10 A.M. on the 22nd, the leucocytic variation was very pronounced. After the attack of vomiting and collapse with fall of temperature, the mononuclear increase gave place to a polynuclear increase, and afterwards continued, but slightly marked, till death on the 29th.

Parasites.—Parasites were present in large numbers before the morning of the 22nd.

22nd. 10 A.M. Large numbers of small forms.
23rd. 8 „ Parasites scanty.
24th. Parasites absent.

Parasites continued absent till death.

20th—24th. Pigmented, large mononuclear leucocytes present. Rare on the 24th.

25th—29th. Pigmented leucocytes absent.

Autopsy.—Icterus was still present.

A peculiar smell of body and organs was noticeable.

A few small pericardial hæmorrhages present.

Gall bladder contained dark green inspissated bile.

Liver much enlarged. Liver and spleen much pigmented.

In spleen films, large ovoid, and round parasites containing much pigment (some of them intracorpuscular), probably related to crescents. Large numbers of much pigmented mononuclear leucocytes.

No parasites seen in brain films.

The tissues have not yet been examined.

Case IV.—S. had not been in the tropics before coming to B.C.A. Came to B.C.A. in 1895. Spent two years in Chinde, on the coast, without having any fever. He then went to Patima, on the lower Shire river, where he has lived since. He has had about six attacks of fever; none very severe.

On June 24, 1899, he came up the Shire river in a houseboat, and was much bitten by mosquitoes. On the morning of July 5 had a shiver, followed by a rise of temperature to 104°. Late the same afternoon his temperature was 99°. Next day he had another attack, and his temperature taken several times during the 7th was 101°.

At 10 P.M., July 7, he took 0·6 gramme of quinine. At 3 A.M. on the 8th (*i.e.*, five hours later), he had a rigor and vomited. At 8 A.M. on the 8th, ten hours after the quinine, passed about "a pint" of bright red, clear urine (Hgb).

He then travelled to Blantyre, and was admitted to hospital. On admission at 10 P.M., on the 8th, urine was not hæmoglobinous.

During the 9th his temperature was normal, and he was feeling quite well. No parasites were present.

At 11 A.M., on the 10th, the medical attendant gave 0·6 gramme of quinine, and 0·3 gramme at 12 noon. Half an hour after the second dose patient had some tinnitus. At 5 P.M. passed some urine deeply hæmoglobinous. The hæmoglobinuria continued until the morning of the 11th. At this time a slight general yellowness of skin and conjunctivæ was noticeable.

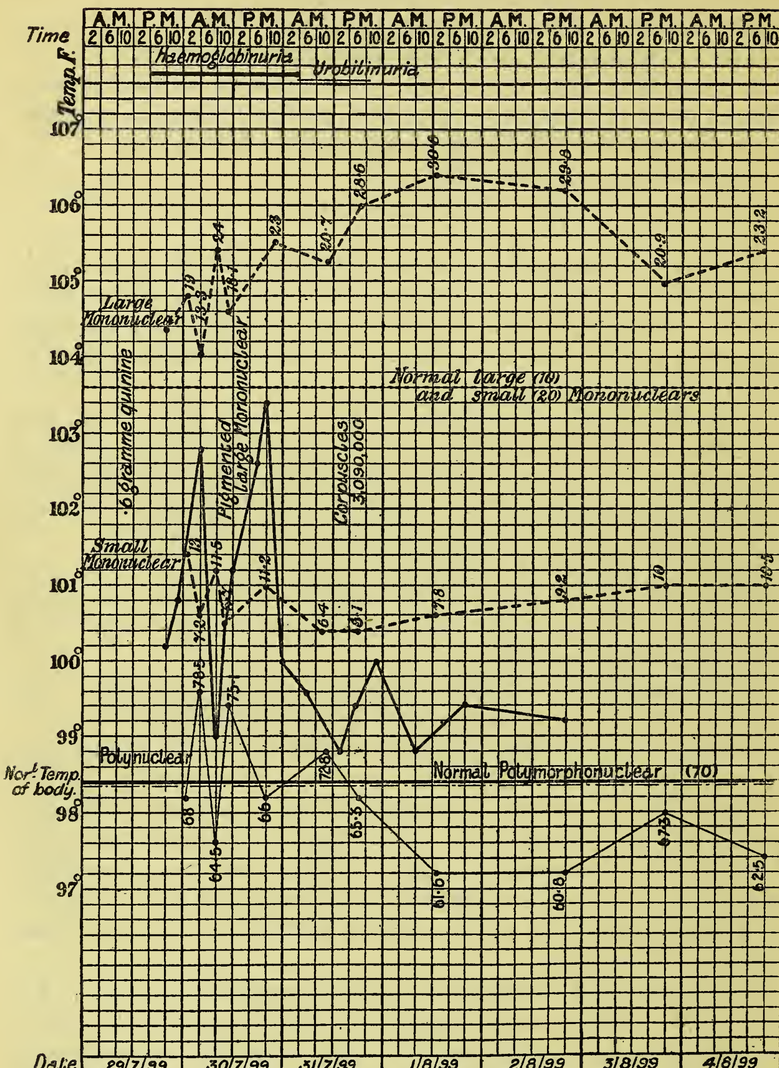
The patient rapidly convalesced, and left hospital on the 22nd. He remained in Blantyre, where exposure to fresh malarial infection was extremely improbable, until the 27th, when he left for the lower river. On the 29th he had a slight feverish attack, similarly on the 30th and 31st. On August 1 had a severe attack of fever, and again came to Blantyre. In hospital at Blantyre he had a severe typical malignant tertian fever, with numerous parasites. He was not given quinine until the fifth day. No hæmoglobinuria resulted.

Hæmoglobinuria and Urobilinuria.—

8th. 2 A.M. to 2 P.M. Mixed urines. Red in colour. No deposit.

Chart IX.

Case IV (*vide* also Chart VIII).



Oxyhæmoglobin bands, reduced by Stoke's fluid; returned on shaking. Warmed with NaHO hæmachromogen bands, probably indicating reducing body in urine. A few minute reddish crystals present (? bilirubin).

8th and 9th. 10 P.M. 8th and throughout 9th, urine contained no hæmoglobin.

All gave marked acid urobilin band.
All contained albumin.
Bile pigment absent in all.
10th. 5 P.M. *Urine* red. Oxyhæmoglobin.
7.30 and 9.30 P.M. Deep brownish-red.
Oxyhæmoglobin bands confluent.

Gmelin's test for bile pigments negative. Also negative applied as follows :—Lime water added to urine. CO_2 passed through precipitate separated by filtration, and dried; test applied to dried precipitate.
11th. 2 and 3 A.M. Deep brownish-red; considerable sediment of urates.
Methæmoglobin.
No bile pigments.
8 A.M. Light, brownish-red. Oxyhæmoglobin much less in amount.
10 A.M. No hæmoglobin. Acid urobilin band.

12th. Urobilin still present, but not marked. No albumin. A nucleo-proteïd reaction marked.

13th. Urobilin absent.

Throughout the case the urine showed no epithelial or coarsely granular casts (absence of kidney changes). A fine brown granular depôsit of urates occurred in urine passed during the second attack of hæmoglobinuria.

Vomit.—On the 11th, vomit dark green in colour. Extracted with alcohol gives Gmelin's reaction for bile pigment.

Blood.—Red corpuscles. Normal in appearance.
3 P.M., 9th. 3,430,000 per c.mm.
5 P.M., 10th. Hæmoglobinuria.
10 A.M., 11th. 2,777,000 per c.mm.
10 A.M., 14th. 3,190,000 „
Leucocytes considerably diminished throughout
3 P.M., 9th. 2000 per c.mm.
10 A.M., 11th. 2000 per c.mm.
10 A.M., 14th. 7000 „
Intra-corpuscular parasites absent. Cultures made from blood on agar remained sterile.

Evidences of a Malaria Attack previous to Admission.—

I. *Pigmented Leucocytes.*—8th, 11.30 P.M., a considerable number of typical pigmented large mononuclear leucocytes were found. These were in subsequent specimens absent, and only isolated grains found in mononuclear elements.

It will be thus seen that no pigmented leucocytes were found during

or after the second attack of hæmoglobinuria, although there was a marked mononuclear increase.

II. *Crescents.*—Single isolated crescents were found after prolonged search on the 9th, 11th, and 12th.

III. *Leucocytic Variation.*—There was a marked increase in the large mononuclear elements on admission, which underwent a gradual diminution. Immediately prior to his second attack of hæmoglobinuria they again suddenly increased in numbers. During the pyrexia accompanying the hæmoglobinuria this was replaced by a polynuclear increase, the mononuclear increase again being marked on the subsidence of the temperature, and diminishing gradually during the next few days (*vide* Chart IV).

Experiments relative to the Isotonic Point of the Blood, and the action of Quinine in Vitro.—

1.

11.7.99.	Salt solutions of the following percentages.					
	0·37.	0·39.	0·41.	0·43.	0·45.	0·47.
Patient's blood.....	h.	h.	h.	H.	H.	No "H."
Normal blood......	h.	h.	h.	H.	H.	No "H."

12.7.99.	0·37.	0·39.	0·41.	0·43.	0·45.	0·47.
Patient's blood	h.	h.	h.	H.	H.	No "H."
Normal blood......	h.	h.	h.	H.	H.	No "H."

2.

	Saturated quinine hydroc. in 1 per cent. salt.	Half dilution.	One-fourth dilution.	One-eighth dilution.
Patient's blood ..	h.	h.	Clumping.	Clumping.
Normal blood ...	h.	h.	—	—

3.*

	A normal serum before taking quinine.	A normal serum after taking 2 grammes quinine.
Patient's blood...	No "h."	No "h."

4.

	Patient's serum.
Normal blood	No "h."
Normal blood after 2·0 grammes quinine..	No "h."

Case V.—Five years' residence in B.C.A. Says he had occasional attacks of fever. Was at Chiromo on the Lower Shire 5.7.99, and remained there until 19.7.99. Was badly bitten by mosquitoes whilst at Chiromo. Returned to Blantyre 27.7.99; rode 20 miles on a bicycle, took 0·6 gramme quinine in the evening; 28.7.99, rode five miles, and took 0·3 gramme quinine; 29.7.99, took 0·6 gramme quinine in morning about 8 A.M.; about 3.30 P.M. passed black water, also at 5.30 P.M.; 9 P.M. was seen in bed; said he felt all right; temp. 100·2° F.

30th. 2.40 A.M. Rigor followed by vomiting and profuse sweating.
9.10 A.M. Rigor followed by vomiting and sweating. Slight icterus noticed.
2 P.M. Admitted into hospital; no further vomiting or complication.

31st. No hæmoglobinuria. Albumin and urobilin present.

1st. Left hospital, though still weak.

Hæmoglobinuria, &c.—

29th. 5 P.M. Methæmoglobin.
8.30 P.M. Methæmoglobin.
11.10 P.M. Methæmoglobin; clearer.

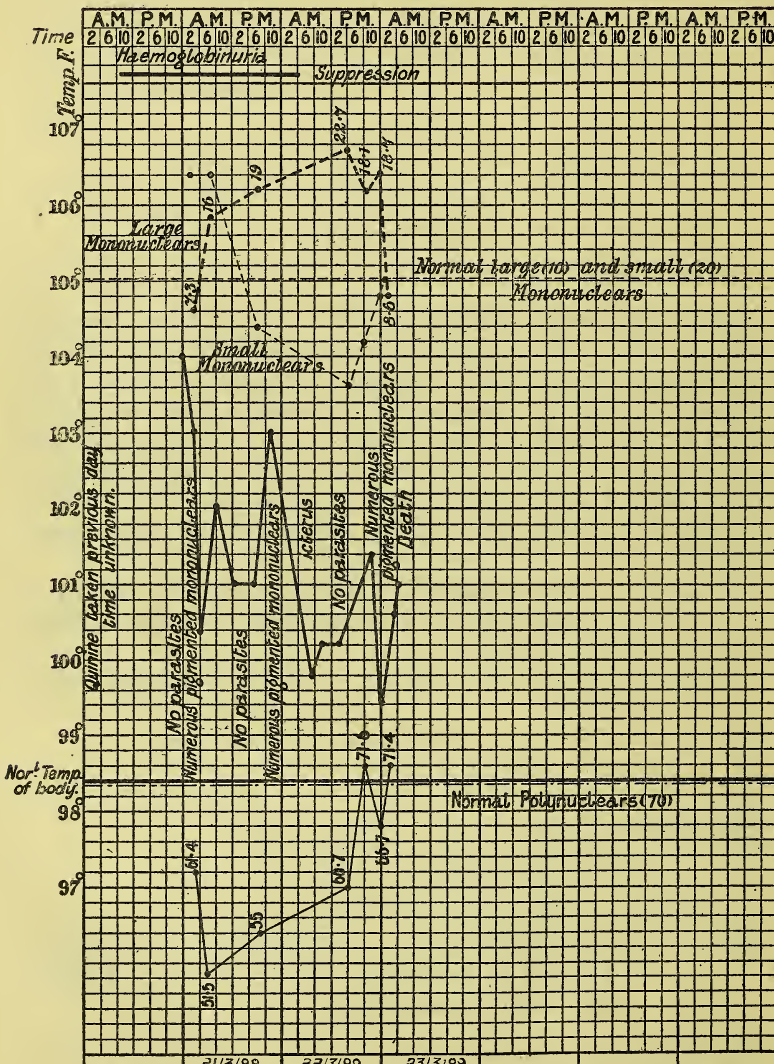
30th. 3.25 A.M. Red turbid urine. Methæmoglobin; not distinct. Some pain on micturition. Urine passed at frequent intervals in average amounts.

* The hæmolytic action of quinine on blood is more evident if a saturated watery solution be used and dilutions made with 1 per cent. salt. In this case the hæmolytic action extends to 4 or 5 dilutions.

7.30 A.M., 10.15 A.M., 11 A.M., 11.40 A.M., all showing methæmoglobin.
8 P.M. Methæmoglobin.
8.30 P.M. Oxyhæmoglobin.

Chart X.

Case V (*vide* also Chart IX).



31st. 7.30 A.M. No hæmoglobin. Albumin and urobilin present.
1st. 11 A.M. No hæmoglobin; no urobilin; no albumin.

The urine at no time showed any evidence of kidney changes; no casts or kidney epithelium. The early specimens contained abundant urate sediment.

No bile pigment in any of the specimens passed.

No quinine was administered during the course of the disease.

Blood.—Corpuscles normal in appearance and colour.

31st. 5 P.M. Red cells, 3,090,000 per c.mm.; leucocytes, 6000 per c.mm.

No parasites were found in any specimen, though frequent examinations were made.

Evidence of malarial infection.—

1. A typical pigmented mononuclear leucocyte was found in the blood taken on 9 P.M. 30.7.99. They were not found in other specimens.
2. The changes in the relative proportions of the leucocytes was of the same character as those we have already described.

The data will be found on the accompanying chart, where it will be seen that there is a mononuclear increase. (Chart IX.)

In five cases of blackwater we have seen that urobilinuria is a constant phenomenon after the occurrence of hæmoglobinuria. In two cases urobilin was present before the attack; of the others we have no data.

It may then be of significance to determine if possible in what proportion of cases urobilin precedes an attack of hæmoglobinuria, or whether a malarial case with urobilin is in any way more likely to become a case of blackwater than one in which urobilin is absent.

A third case, not reported here, came under our observation.

A patient, in hospital with malaria and urobilinuria. Out of hospital about a fortnight later, he had blackwater fever (after quinine). On his return to hospital again in about a fortnight, malaria (many parasites), urobilinuria.

CHARTS.

The figures in the rings show the sizes of the parasites, *e g.*, 3 means that the parasite is one-third the diameter of the red cell. The perpendicular columns and the figures at their head show the number of parasites at a given time, *e,g.*, 1·0 means that an average field contained one parasite and 0·1 that ten average fields contained one parasite.

The leucocytic curve is constructed in the following way. The base line for the large and intermediate mononuclear leucocytes represents a percentage of 10 (a high normal value), while that of the polynuclear represents 70 (a low normal value). Thus in Chart V 31·1 signifies that the mononuclear leucocytes are 21·1 per cent. above normal. The figures represent the average deduced from over

1000 leucocytes counted. It is advisable to have large surfaces of blood evenly distributed. For this purpose by far the best preparations are to be made by distributing the blood on a glass slide by means of the flat end of another slide (it is necessary to ascertain that the slides fit one another, as a slight curvature is often present), or by simply using a needle. Cigarette paper used for this purpose gives extremely bad films.

The large mononuclear value includes also the intermediate cells, while with the polynuclear are included cells of a transitional type.

Chart I.—Exemplifying tertian character of fevers.

Chart II.—Showing continuance of fever, in absence of parasites due to taking of quinine; also the large leucocytic variation.

Chart III.—Showing continuance of fever in absence of parasites and a late development of the leucocytic variation.

Chart IV.—Showing leucocytic variation and its connection with the temperature curve; the mononuclear elements increase with the fall and the polynuclear with the rise of temperature.

Chart V.—A further example; the variation is well marked.

Chart VI.—Showing great mononuclear increase throughout.

Chart VII.—(Case 3.) Blackwater fever. A typical malarial chart with marked leucocytic variation; sudden interruption, coincident with pernicious symptoms; collapse; administration of quinine; disappearance of parasites; hæmoglobinuria; death.

Chart VIII.—(Case 4.) Blackwater fever. Taking of quinine; hæmoglobinuria; absence of parasites; presence of pigmented leucocytes; leucocytic variation; recovery.

Chart IX.—(Case 5.) Blackwater fever. Quinine hæmoglobinuria; absence of parasites; pigmented large mononuclear leucocyte; leucocytic variation; recovery.

Chart X.—(Case 2.) Blackwater fever. Quinine hæmoglobinuria; absence of parasites; pigmented large mononuclear cells; leucocytic variation; death.

"Distribution of Anopheles in Sierra Leone. Parts I and II." By J. W. W. Stephens, M.D. Cantab., and S. R. Christophers, M.B. Vict. Received April 5.

Part I.—*Freetown.*

The Malaria Expedition from the Liverpool School of Tropical Medicine* visited Freetown during the months of August and September. They found two species of anopheles (large and small) in abundance, and in two institutions, where there were at that time many cases of malaria, about 25 per cent. of the anopheles had sporozoites in the salivary glands or zygotes in the median intestine. They also found that anopheles bred exclusively in small pools scattered throughout the town; and in a report to the Governor of Sierra Leone they considered that an extermination of anopheles was possible

* *Editorial,* 'British Medical Journal,' Sept. 9 (p. 675) and 16 (p. 746), 1899.

by destroying the larvæ in these pools. Following the recommendation in this report, the sanitary authorities of Freetown applied tar regularly for a period of some months to these pools, but as soon as the tar applications were discontinued the anopheles reappeared everywhere in their old breeding-places.

Freetown occupies a strip of gently sloping ground between the sea and the base of steep, densely wooded hills which lie behind it. The strip is composed of lava rock (laterite), and though in places a thin covering of earth is present, yet the greater part of the surface of the ground is formed by an outcropping of the solid rock, the untreated surface of which forms many of the streets of Freetown. The rock weathers so as to form a very uneven surface, and small hollows capable of holding water are very numerous. It is in portions of the town built upon the bare rock that the great majority of the puddles described by the expedition existed during August and September. The central portion of the town in which most of the Europeans reside is built upon clayey earth, through which the rock only occasionally protrudes. The roads are here even, and for the most part have at their sides open triangular drains for surface waters, which if well made do not allow pools to form. In this district only an occasional anopheles pool is noted in the map of the expedition.

Both the east and the west portions of Freetown, however, have large areas where the streets are formed by the natural rock, and if drains exist they are ditch-like and flat-bottomed. In these, pools readily form if there is not a constant flow of water. Such an area of about half a square mile in extent exists in the western portion of the town, and contains the majority of the pools described by the expedition. Another area, not so extensive, in the eastern portion of the town almost completes the number. Both districts are among the most densely populated portions of Freetown.

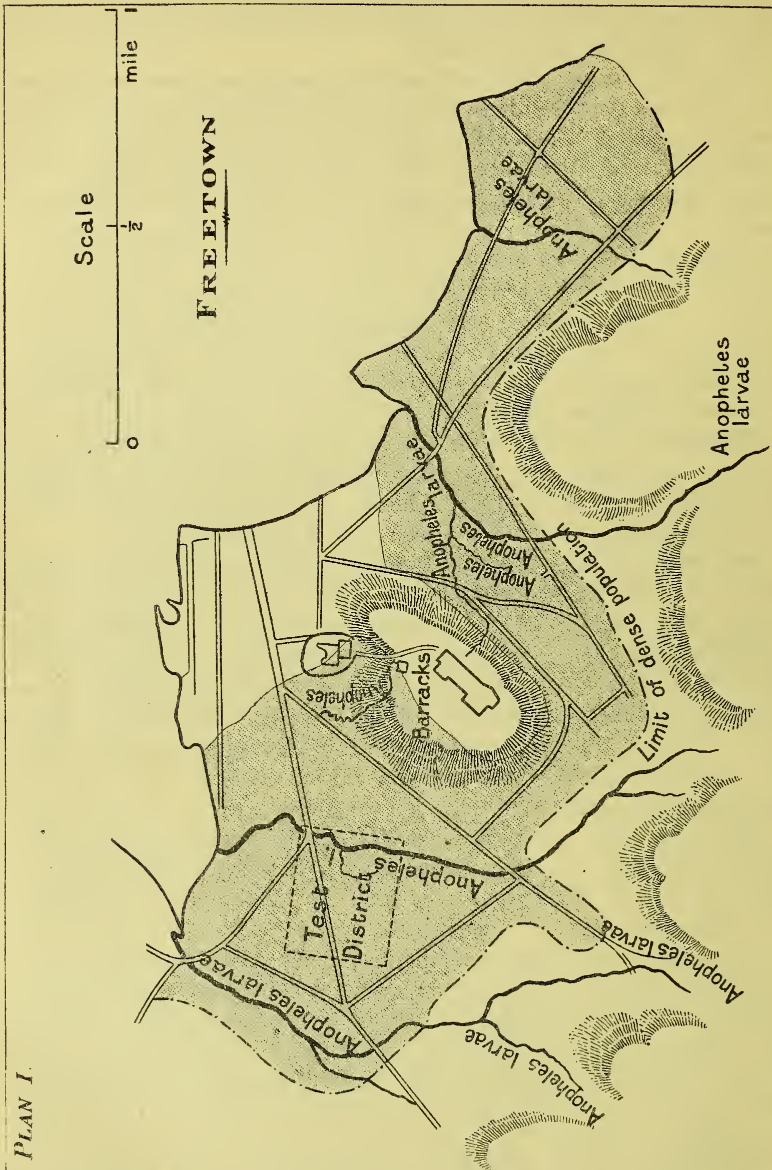
The main rains in Sierra Leone fall during a period lasting from June to October. It is in these months that the puddles exist, as described by the expedition. But from the statement of the sanitary authorities and from our own observation the pools at the end of the rainy season are more numerous than represented. It is in these months that most of the sickness occurs among the Europeans resident in Freetown. From October to the end of March rain is absent, and the soil becomes uniformly parched, and it is a striking feature that during these latter months very few cases of fever occur in Freetown. This holds good both for the Europeans and for the West Indian regiments.

Coincident with this decrease in malaria there occurs a diminution in the numbers of both the breeding-grounds and the adult insects of anopheles.

During December there is a drying up of all the natural waters in

the town. This is hastened during the latter part of January by the Harmattan—a dry wind from the interior. By the end of January

Distribution of Adult Anopheles in the Dry Season.



The shaded area represents the portions of the town where winged anopheles are found with great difficulty, but are sufficiently numerous to restock any freshly formed pool. The clear areas are comparatively free from anopheles.

the rocky areas were, with rare exceptions, free from the pools noted in the map of the expedition. And in the dry season, as we shall see, it

is not in these pools that we have the main source of anopheles in Freetown.

Certain small streams arise from the hills behind Freetown, and flow in their natural channels through the town. Of these, two especially are to be noted—Saunders' and Nicol's brook, situated in the western and eastern portions of the town respectively. These streams in the dry season for varying distances flow over beds of bare irregular rock, forming numerous small rock pools, either wholly or partially detached from the running water. In these, anopheles larvæ were found in abundance. They occur not only in the pools but even in the back eddies and sluggish corners of the stream. We have traced these streams backwards far up into the hills; and here, in the centre of dense bush, wherever a rocky bed allowed of the formation of an isolated pool or back eddy, innumerable larvæ were to be found. A similar condition exists in all the hill streams in the colony of Sierra Leone. Wherever the water can form a small isolated pool, as in a rock hollow, innumerable larvæ existed.

Saunders' brook, which runs through the most thickly populated and most squalid part of Freetown, passes over rock for half a mile of its course, and swarms with anopheles larvæ from December onwards.

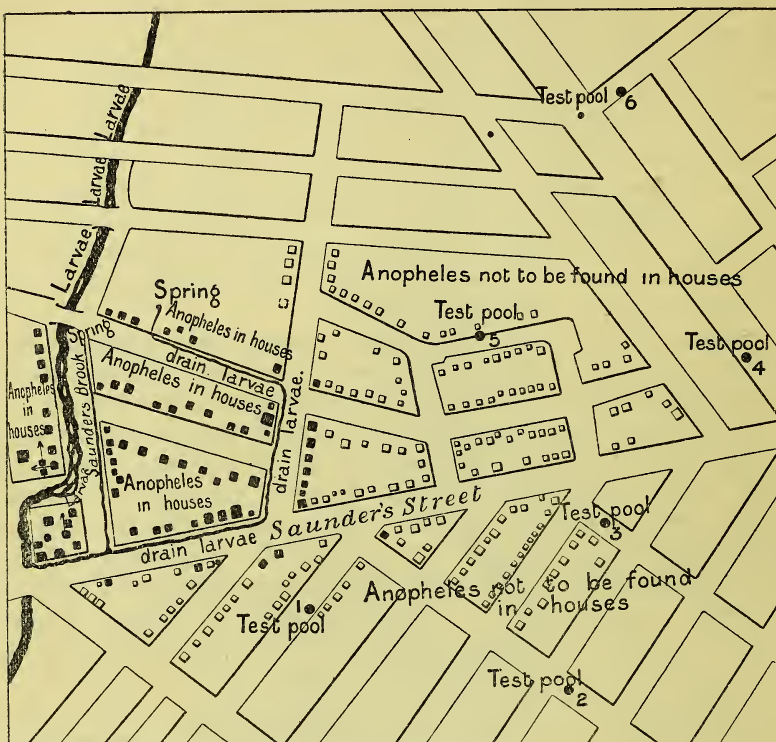
Besides these somewhat considerable bodies of water, anopheles were found to be breeding in several small runnels of water, which were kept from drying either by small springs in the town or by waste water from the military barracks. These small waters are few in number, but usually traverse long and indirect courses before reaching their destination, and are a fruitful source of anopheles from December onwards. At the end of the dry season they are, however, for the most part dry. In no other situations did we find anopheles larvæ in Freetown than in the brooks and small drains.

Considering the rock puddles throughout Freetown alone, we see that they become dried up during three months in the year, and thus cease to serve as breeding-places. This drying up is brought about through natural agencies, which are evidently more effective than any artificial measures which might be resorted to. Nevertheless, anopheles reappear in undiminished numbers in these areas after the onset of the rainy season.

An investigation of the means by which such a restocking of the town by anopheles takes place at the commencement of the rains seemed to us, then, of importance, and a knowledge of this point was necessary before one could form an opinion as to the probable result of measures taken to destroy larvæ in these pools.

A representative district of the town was therefore taken which included a portion of Saunders' brook, a surface drain about 200 yards in length fed by a spring, and a strip of rocky ground which contained numerous pools in the wet season but none in the dry. (See plan.)

In portions of the test district remote from the brook, anopheles were, with rare exceptions, not found by a most careful search in the early morning in very many houses; and among the numerous mosquitoes brought to us by the inmates they were only very rarely present. In the neighbourhood, however, of the brook and drain, anopheles



PLAN II. TEST DISTRICT.

Explanation:—

- ←■ *Houses in which anopheles are present in enormous numbers.*
- ■ *Houses in which search in early morning reveals a few anopheles.*
- □ *Houses in which anopheles cannot be found.*
- ● *Test Pools. 1,4,5, are natural rock hollows; 2,3,6, are cement-made hollows*

were to be detected in most of the houses. But even here, in well-built, clean houses, it was difficult to obtain them in the early morning, such anopheles as entered at night appearing not to remain. In small dirty and dark houses, however, a variable number were always to be obtained. Here and there in the district bordering upon the stream were certain thatched sheds in which native boys slept. The inmates of these lodging-houses numbered as many as twenty, all

sleeping upon the ground in a space of about 1500 cubic feet. These overcrowded sheds were especially the abode of anopheles. In such places female anopheles were found in large numbers, while in houses immediately adjoining they were difficult to obtain. Males were also to be found, but much less frequently than the females. We would draw especial attention to the fact, of which we shall give further instances, that in native dwellings—and in these overcrowding is often extreme—anopheles are frequently present in enormous numbers. Out of seventy anopheles examined from one of these sheds five contained glands crowded with sporozoites, and one contained two small zygotes. An examination of the blood of sixteen of the inmates did not reveal any crescents. Nor was this exceptional, for in native quarters we have frequently found a percentage of anopheles with sporozoites, though cases of fever could not be discovered. In all cases of anopheles from native quarters examined by us the sporozoites have been greatly in excess of the zygotes, and it is probable the sporozoites remain unchanged for considerable periods in the glands. Nor does feeding apparently exhaust the supply of sporozoites, for the glands of freshly-fed mosquitoes are only in part emptied of secretion, and portions of the glands still contain numerous sporozoites.

We found, then, in the dry season along the course of the streams an unlimited supply of anopheles; and in the houses adjoining, anopheles were found in numbers under the above-described conditions, whilst at a distance from the streams anopheles were rarely to be obtained. The view then seemed possible that in these streams we had the origin of the mosquitoes which reinfest all the pools during each rainy season. But there was another possibility, viz., that mosquitoes remained concealed in grass, shrubs, and trees during the dry season ready to lay their eggs on the first formation of suitable pools. To test this possibility the following experiments were devised:—A series of small artificial pools were made in different parts of the test district. Some of these were small natural hollows in the rock, others were formed with cement. The position of these pools is shown in the plan. They were pools selected as far away as possible from Saunders' brook. These five pools were kept supplied with water from the tap supply of the town. They all without exception after some days contained minute anopheles larvæ. The experiment was repeated with an identical result. In one pool the experiment was performed three times with the same result. It is an interesting point that the occurrence of culex was the exception not the rule in these pools.

Control pools covered with mosquito net remained free from anopheles larvæ.

That both the pools formed in the receptacles made of cement and the twice filled natural rock hollows were infested shows that adult

anopheles themselves are present throughout the dry season, and that the result was not due to the development of ova.

It is very unlikely that ova should be able to withstand the intense heat of the sun upon the rock for so many months. We have found that desiccation on blotting paper for twenty-four and forty-eight hours respectively did not prevent subsequent hatching. Further desiccation, however, did so. It is possible that the natural conditions were more favourable than artificial drying. The earth from twenty-five dried up anopheles pools was therefore collected, and the effect of adding water in shallow trays observed. In no instance did larvæ develop. The experiment then confirms our deduction from the fact that larvæ were found in pools made of cement only.

Although it is almost impossible to find anopheles in houses in the dry season, this experiment proves their continued presence in the district. So that we have here alone a source of anopheles amply sufficient to restock with larvæ innumerable pools during the rains as all of our artificial pools were stocked. In fact, we consider that in a large part of Freetown, especially the outskirts, the conditions are parallel with those which we shall describe in Part II as characteristic of the bush. We can hardly hold that the anopheles were derived from the streams at this season of the year, for the uniformity with which the artificial pools became infected negatives this view.

The fact, then, that at the end of three months in this area, which was free from breeding-grounds, anopheles are still to be found ready to lay their eggs is an important one, and the negative result of the experiments of the sanitary authorities becomes easily explicable, and, indeed, might have been predicted.

If we now summarise the sources of anopheles in Freetown, they are—

1. The streams (larvæ).
2. Certain small drains (larvæ).
3. Rock pools (larvæ), in the rains only.
4. The gardens (mosquitoes).
5. The houses (mosquitoes), especially where overcrowding is present.

Before dealing with the large question of the destruction of anopheles, we will first describe our experiments made with larvicidal substances. We selected as the most practical larvicides paraffin oil and salt. These, as it appears from Celli's and Casagrandi's experiments, are the most efficacious of substances, easily and cheaply obtained. Thus petroleum 0·2 c.c. per 100 sq. cm. was found by them to kill larvæ and nymphæ in four hours; saturated salt in thirty seconds; milk of lime, 5 per cent. in forty-eight hours.

The action of paraffin as a larvicide was tried upon—

(*a*) Rock pools,
(*b*) Small runnels of water.

The larvicidal effect in the pools was very striking, most of the larvæ being killed in fifteen minutes, or less. In many cases, again, besides the larvicidal effect, adult females were found next day killed by the paraffin on the surface of the pool, where they had come to lay their eggs. The final result was, however, the same as that experienced by the Freetown authorities, *i.e.*, immediate return of the insects on the cessation of the application of petroleum.

As a test experiment on small bodies of running water, a surface drain was selected arising in a spring not far removed from Saunders' brook, and, after a course of about 300 yards, flowing into this brook. The spring was free from larvæ, and we have rarely found anopheles larvæ in any spring of pure water. The drain, however, teems with larvæ throughout its whole length. Over this drain kerosine oil was sprinkled by means of a watering-pot. About four gallons sufficed to cover the drain thickly with oil. The larvicidal effect was immediate, and on the following day no living larvæ were seen. Four days later traces of oil were still present in places. Eight days later small larvæ were present along the whole drain. In our experiments with paraffin the oil was intentionally poured on in sufficient quantity to spread readily over the pool, so that no larvæ or nymphæ might escape, but in spite of this, so soon as the paraffin evaporates the pools become infested. We have seen, however, that both in pools and drains, paraffin oil at the time of its application is both a certain and swift destroyer of larvæ and nymphæ. A weekly application of paraffin, then, would effectually prevent the formation of the perfect insect in these situations.

In experiments made, it is true roughly, by throwing a few handfuls of salt into pools containing not more than three or four pints of water, no larvicidal effect was produced in three days. No doubt by making the resulting solution stronger a better result might have been obtained.

If we now turn to the question of the destruction of anopheles, we must bear in mind the conditions we have described above. The problem is a difficult one, but the principle which should underlie all such endeavours is essentially that of drainage. Every endeavour should be directed to the prevention of small collections of standing water.

1. We have in the streams a large extent of water, even at the end of the dry season; in the rains these are rushing torrents.

2. We have in the rock pools a series of small collections of water, spread almost over the whole of Freetown, with the exception of the central portion. During the heavy rains they must be even more numerous, while in the outskirts of the town they become innumerable.

3. About six small drains or runnels of long and devious course, already described.

e

These latter could easily be reconstructed so that the water would flow in triangular channels, or even pipes, and their removal as sources of anopheles would be very beneficial.

The second source of water could, we think, be dealt with more readily, and with less expenditure than the first.

It would not, we think, be difficult to convert the irregular rocky roads in the outer portions of Freetown into roads of an even surface of earth, such as exist in the central portions of the town; nor to construct stone gutters of triangular section throughout the whole of districts now without any surface drains, or with only badly-made and ill-planned ditches, which advance rather than retard the breeding of anopheles.

The difficulties in the treatment of such varying quantities of water as flow in the streams in the dry and rainy seasons respectively we are unable to estimate; but these streams, as we have pointed out, are the main source of anopheles in the dry season, and effective treatment of such a large source ought on no account to be omitted.

No doubt in the absence of constructive works for improving the surface drainage a thorough application of paraffin oil to the pools at short intervals would form, so long as continued, a check upon anopheles. Such a procedure to be efficient, however, must be very thoroughly prosecuted, and we feel sure could not be carried out without considerable expense. Moreover the expense would be a yearly one, as we have seen that immediately operations cease anopheles become as abundant as ever, nor, in the absence of any radical treatment of the streams, could the result be anything but partial.

We have preferred, then, the radical treatment of the rock pools, viz., that involved in levelling and surface drainage, to attempts at destroying the larvæ, even by such an efficient agent as paraffin, though no doubt paraffin would be very useful as a supplementary agent.

That such levelling and drainage would be effective is rendered highly probable by the condition of the central portion of the town, which only differs from the eastern and western portions in the possession of even road surfaces and triangular drains. Here in the dry season anopheles are not to be discovered in the houses, and test pools, similar to those used in the test district and described above, do not become so readily infected. Also in the rains the malaria expedition has marked not more than two or three anopheles pools in this district. We should endeavour, then, to extend more and more this district of comparative freedom from anopheles.

We have seen further that anopheles are prone to collect in small, dirty, overcrowded houses, and that they remain throughout the dry season, very probably hiding in the daytime among the vegetation, of which there is a considerable amount in Freetown. Such factors

should not be neglected, and the removal or regulation of overcrowded hovels, and the clearing away of rubbish and useless vegetation, must have considerable effect upon the general result.

We must refer finally to a condition which we shall describe in detail in Part II of this report. In a small clearing in the bush, on the Sierra Leone Government Railway, are situated two small wooden houses, and adjoining them small native huts, five in number, occupied by the native servants. The country for a quarter of a mile in every direction consisted of dry bushland. Here at the time of our visit, extending over some weeks, nowhere could we find culex or anopheles larvæ. Yet in these houses anopheles and culex were present, and in the native huts they were present in large numbers. Although in the bush itself anopheles were only rarely to be caught at night, yet in the compound the numbers were very great. We must emphasise again this peculiar fact, that in a district where apparently anopheles may be rare, they aggregate in quantity if dirty native dwellings or villages are contiguous. The danger of this condition to the Europeans inhabiting the wooden houses is a very real one, for all the inmates of the dwellings were continually suffering from fever.

Our object, however, in drawing attention here to the condition of things we shall describe fully in Part II, is the following :—

We believe that there is some intention of constructing a light railway in Freetown, and building European residences on one of the adjacent hills. This on general grounds should be most beneficial, but unless the conditions we have described above are appreciated, we cannot expect that it will have the effect of appreciably reducing the amount of malaria. For here we still have a parallel set of conditions to those we have just described. A house built on the top of a hill surrounded with bush, and with its adjoining native huts, will be no freer from anopheles than a house in Freetown itself. The conditions, in fact, will be identical with those at Kissy Asylum and the Wilberforce Barracks, where infected anopheles were readily found by the Liverpool Malaria Expedition.

If this scheme, then, be carried out, the following points must be adequately considered :—

1. The necessity for extensive clearings around the houses, and the prevention of the formation of pools in the clearing.

2. The necessity for cleanly native quarters, and avoidance of overcrowding.

3. The almost invariable presence of anopheles in mountain streams and rock pools.

We may formulate our conclusions in the following way :—

1. In the dry season, in the absence of pools, anopheles breed freely in streams and drains.

2. Anopheles exist in most parts of the town, excepting the central

area, throughout the dry season, ready to lay their eggs when pools appear.

3. Overcrowded native huts and native quarters generally are usually infested with anopheles, and a source of danger to neighbouring houses.

4. That the only adequate method of treating streams, drains, and rock pools is by surface drainage, and the use of well-made triangular gutters.

5. That the destruction of rank vegetation, the destruction of dirty huts, and the prevention of excessive overcrowding in these will contribute largely towards the general result.

Considering what a scourge malarial fever is, and how definite is its cause, no effort should be spared to rid the town, or at least diminish the number, of anopheles that now abound there. The result can almost certainly be effected, but the means adopted must be commensurate with the magnitude of the task.

Part II.—*The Bush.*

1. Immediately outside Freetown we find hills covered with dense bush. These constitute the greater part of the colony of Sierra Leone proper. Throughout the whole of the district anopheles larvæ were present. In the mountain streams wherever there were suitable pools multitudes of larvæ existed. In tracing these mountain streams occasionally for half a mile or so we have found no larvæ, but then a rocky pool occurred, and here they were again found in numbers. As we shall eventually show, the conclusion is not justifiable that where there are no pools with larvæ there are no mosquitoes. Pools simply form a convenient means of detecting their presence, which otherwise may not be easy.

In Freetown we showed how by constructing artificial pools the presence of mosquitoes, otherwise apparently absent, could be detected. Similarly, on the summit of Sugar-loaf Mountain (about 2000 feet), the presence of a small pool with larvæ revealed the presence of anopheles.

The expedition of the Liverpool School of Tropical Medicine found at Wilberforce Barracks, an institution situated upon the hills at a considerable height above sea level, very numerous anopheles in spite of the fact that no breeding-places were apparent. At Kissy Asylum, also situated on the hills and surrounded by bush, a similar condition of affairs existed, namely, numerous adult anopheles without any apparent source.

We shall show, however, that this is no exceptional but a general condition that in the bush, in the absence of breeding-places, anopheles

exist, and, under conditions which we shall describe, often in enormous numbers.

2. In this portion of the report we shall, however, describe more particularly the distribution of anopheles in the districts of the interior, which to a large extent consist of low bushland intersected by extensive swamps. It is here that the colonist, trader, or missionary is often located, and it is country of this description that is traversed in expeditions, military or otherwise, and it is through this bushland that roads and railways are made; it is here that temporary camps are pitched or clearings made, and under such conditions especially as is well known malaria is contracted. A knowledge of the distribution of anopheles, then, is of the utmost importance.

The observations recorded in the present paper refer to the distribution in the dry season. Considerable differences occur in the rains, and the conditions at that time we shall record later.

One hears surmises as to how malaria can be contracted in districts rarely visited by man. In our experience, such conditions are rarely fulfilled, and we shall show how very probable it is that, in the vast majority of cases where men have been supposed to have contracted malaria in uninhabited swamps, they have in reality become infected in houses, camps, or native villages where fever was pre-existent.

At the time of our stay in Sierra Leone an extension of the Government railway was in progress. This afforded us an opportunity of investigating the conditions under which the operatives contracted malarial fever. During the last four months a number of men have been employed in constructing a bridge across the Ribbie River at Mabang, in the midst of an extensive mangrove swamp. Among them malarial fever, as the accompanying chart shows, is almost constantly present. The universal opinion was expressed that their fever was due to working in the fœtid mud of the mangrove swamp, but we shall show that this is not so, and that almost certainly the malaria has been contracted in their houses, built upon slightly elevated and perfectly dry sites. The men's quarters are at Songo and at Mabang.

At Songo there are six houses (numbered in the chart 1 to 6). Of these, 1, 2, and 3 are short distances apart, 4, 5, and 6 are close together in the same compound, and may, so far as anopheles are concerned, be considered as a single house.

At Mabang there are three houses, situations of which are shown on the accompanying plan. Of these, those numbered 7 and 8 in the table are in the same compound, whilst 9 is half a mile away. The occurrence of malaria among the occupants of these houses is shown in the accompanying table. The table shows the house in which the case occurred, and also the week in which the attacked commenced. The figures 2 or 3 denote the number of attacks in the same house.

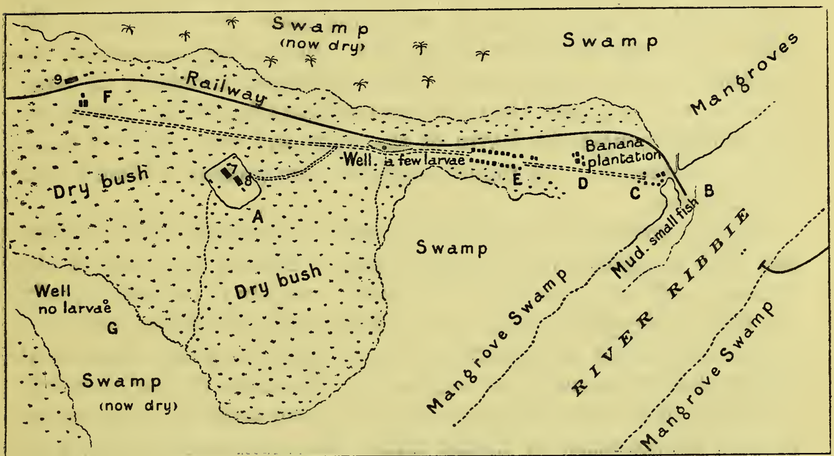
Owing to the absence of records of slight attacks, and from the fact

	July.				August.					September.				October.			
Weeks........	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Songo—																	
House 1	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..
„ 2	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..
„ 3	1	2, B	..	1	2	..	..	..	..	..	..	..	..	..	..	..	..
„ 4, 5, and 6	..	1	3	..	..	1	..	..	..	..	2	1	1	2	2	2	..
Mabang—																	
Houses 7 and 8*	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	1
„ 9	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..
Camp 1	..	..	1	1	2	1	..	1	..	..	1	..	1	..	..	..	..
„ 2	..	..	..	1	..	..	..	..	..	..	..	..	..	..	1	..	1

	November.					December.				January.				February.	
Weeks........	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Songo—															
House 1	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..
„ 2	..	..	B	..	..	..	1	..	..	..	..	..	..	..	1
„ 3	..	1	..	..	..	..	..	1	..	..	..	..	..	..	..
„ 4, 5, and 6	..	2	..	1	..	..	.	2	1	..	..	B	..	..	..
Mabang—															
Houses 7 and 8	..	..	..	..	..	..	..	..	2	1	1	3	2	..	1
„ 9	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..
Camp 1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
„ 2	2	..	..	..	..	1	1	..	..	..	..	..	..	..	..

B signifies a case of blackwater.
The lines beneath the figures draw attention to periods when the occupants of the houses were suffering constantly from malaria.
* Houses occupied from the second week in October.

that relapses occur, and also from the probability that mosquitoes infected with sporozoites may remain in native quarters for considerable periods before again infecting a European, it is difficult to determine the period intervening between the transmission of malaria from one inmate to another. The table shows, moreover, that it is usual for houses to have a constant succession of cases, lasting six weeks or more, whilst periods of health intervene. Thus in houses 4, 5, 6, in August and September, ten separate attacks of malaria occurred. In house 3 during July seven cases occurred, and in houses 7 and 8 in January most of the inmates had malarial attacks—nine cases in all.



PLAN OF MABANG.

The condition of these houses at such times we were enabled to study in the case of the last of these outbreaks. The plan shows the chief physical features of Mabang. At "B" the men are working surrounded by fœtid mud in the midst of a large swamp. At the time when, however, all the inmates of houses 7 and 8 were suffering from malaria, those working in the same place, but living at house 9, did not so suffer.

At "A," houses 7 and 8 are situated in a small clearing in the bush, upon a slightly elevated site surrounded at distances of from a quarter to a half a mile by large tracts of swampy ground.

The clearing at "A" lies on porous ground, and being slightly elevated is, except in the rains, free from water. At the time of our visit in January and February not only was the compound itself free from any water, but the bush for a quarter of a mile around was without a trace of water or even humidity of the soil.

In spite of this, in the compound mosquitoes were present in abundance. Two species only were found, a culex and an anopheles.

The culex was a species widely distributed in Tropical Africa, and frequenting chiefly swamps and plains of rank grass. On certain nights they could be captured in large numbers, far exceeding those of anopheles caught at the same time. In the daytime, however, the numbers remaining in houses or tents were small, and less than those of anopheles, a fact which is accounted for by the greater readiness of the culex to leave houses in the morning for the bush.

Anopheles were also present in the compound. In the two houses of the Europeans they were present only in small numbers, though such as were found were generally within the mosquito nets.

Within a few yards, however, of each house there were several small hovels built of palm leaves in which the native servants lived. Of these there were five in the compound. In striking contrast to the condition in the larger houses, these contained anopheles in large numbers, and in spite of the numerous facilities afforded the mosquitoes for hiding amongst the leaves of the walls and roof, numbers could always be obtained. At night, anopheles were present in these hovels in multitudes.

We may notice here a statement sometimes made that natives suffer less than Europeans from mosquito bites. This is true only of the irritation following the bites. A native certainly is not only equally but far more frequently bitten than a European. Nuttall, in his *résumé* of the *rôle* played by insects in disease, cites King and Laveran as stating that the immunity of negroes may be due to the fact that they are bitten less frequently either from an odour noxious to mosquitoes or from the possession of thicker skins. That natives do not enjoy an immunity from mosquito bites is very certain; they, on the other hand, attract mosquitoes, and especially anopheles, more readily than Europeans do.

We have already noted in Part I how in overcrowded native huts there is a swarming of anopheles. This is not exceptional; in nearly all native villages anopheles are to be found by search among the thatch of the huts, and if not within, where it is usual to burn fires constantly, at least under the eaves. At C and D, Mabang village, and at E, where the native workmen on the railway have their quarters, and at F, a frontier police station, anopheles were to be found in extraordinary numbers.

That natives powerfully attract anopheles was well shown in the following experiment. In a tent in which a European had been accustomed to sleep, pitched in the compound at "A," only one or two anopheles were usually to be found in the morning. Two natives were then allowed to sleep in the tent, with the result that the first morning nineteen anopheles were captured. The second morning sixty-two anopheles, most of which had fed, were caught. The natives did not complain unduly of mosquitoes. The use of the tent by the natives

was then discontinued, and the anopheles rapidly became fewer in number.

It is well known that the immunity of natives to malaria is by no means absolute, and even severe cases are frequently to be seen, chiefly in children and old people. And, indeed, we have very constantly found a proportion of anopheles from native villages and quarters to contain sporozoites. The proportion is by no means so large, however, as Grassi has found in the Italian malarial districts in the autumn. Very possibly, however, a similar seasonal prevalence of infection is found in the anopheles in Africa. The number of infected specimens in the dry season varied in different localities from one in five to one in twenty or more.

At both A and D a proportion of the anopheles were infected, the numbers at A varying on different occasions from one in five to one in ten.

At Mabang, then, ample cause for the existence of malaria was found in the presence of infected anopheles in the houses where those men suffering from constant malarial attacks lived.

It is evident the native dwellings are fertile sources of anopheles, and the Europeans in every case had such sources within a few yards of their houses.

It is evident from the table of cases given that when once the anopheles in these situations become infected, the infection is kept up for many weeks or months. We may look upon such a house and its accessory hovels as one infected with malaria, or as a "fever house." Such is the universal condition of European houses, indeed, in the remote stations situated in the African bush. It is the condition under which the coffee planter lives in British Central Africa, and the so-called rest houses and river stations of the various companies reproduce the conditions which we have described, and anopheles were found in all.

It is in such houses that the malarial cachectic is living exposed to frequent or even constant reinfection, and in which every traveller staying the night is liable to infection.

From such fever houses the majority of our cases of blackwater have come, and that such should be the case is strong evidence in favour of the malarial origin of tropical hæmoglobinuria.

It has been noticed that the cutting down of trees has apparently been the cause of outbreaks of malaria, and that in Assam and Borneo it is newly-made clearings which especially give rise to malaria. These observations are borne out by the conditions at Mabang, for although anopheles were to be found in unlimited numbers in all the clearings in which dwellings were situated, yet in the surrounding district anopheles occurred very scantily. There is, then, a marked concentration of anopheles whenever a clearing with human, and especially native, dwellings occurs.

In spite, however, of the occurrence of anopheles at A, C, D, E, and F, yet at neither of these places were any breeding-places discovered. Throughout the whole district shown in the plan, anopheles larvæ were found only in a small puddle by the side of the railway in a pit from which earth was being dug out. This puddle contained only a very few larvæ. Such a source was quite inadequate to keep supplied with anopheles the native quarters at A, C, D, E, and F. Close to the village at C there are extensive areas of mud which at low river are covered with small pools of water. Prolonged search in these, however, failed to demonstrate a single larva. That larvæ were not found in such a situation within a few yards of houses containing the adult anopheles in abundance is interesting. The situation is one that has been usually considered essentially malarial, a creek lined with mangroves and with banks of fœtid mud. The pools are subject, however, to daily disturbance by the ebb and flow of the tidal river water, and small fish abound in them—two factors probably sufficient to explain the absence of larvæ.

The conditions in the compound at "A" were even more striking. In every direction for several hundred yards the ground, which was covered with low bush, was quite dry. Search was made for larvæ in the most minute traces of water, such as collect in the axils of banana and other large leaves, but though at C numerous larvæ of a species of culex were found in such situations, those of anopheles were never encountered.

At "A" then, although anopheles abound, yet for some months there could have been no local breeding-ground; the same holds good for C, D, E, and F, except that, unlike "A," here there are swamps in much closer proximity.

It is an important question to what extent swamps are breeding-grounds of anopheles. Search in an extensive swamp for larvæ is difficult, and that larvæ are not found cannot, we think, be considered good evidence against their existence in limited numbers.

We have frequently captured adult anopheles in swamps remote from human habitation, though in such situations they do not seem to occur in such numbers as in clearings and native villages. Thus, in the small dry swamp at "G" anopheles constantly occurred, though in small numbers. We have also captured adult anopheles several miles from any hut. At both Songo and Mabang, however, we have been able to detect anopheles larvæ in swamps. In the main swamp water we have never succeeded in finding them, and the innumerable small fish present in the swamps would probably prevent their being present in any number. They were, however, occasionally observed in small isolated pools on the mud, and they are still more common in small pools at the edges of swamps. But it is a noteworthy fact that the larvæ of anopheles do not occur in swamp pools in such numbers

as in the streams and rock pools among the hills of Sierra Leone. These rock pools would appear to be the most suitable conditions for the breeding of anopheles.

While at Mabang we examined culex which had fed on human blood, and were caught in the native huts at the same time as anopheles. These likewise contained sporozoites in their salivary glands in the proportion of about one in ten. They were found also in unfed culex caught in the bush, and several infected ones were found in the swamp "G," though here the number of sporozoites was always small.

Sporozoites were also found in two out of four specimens of a large species of anopheles found rarely in the bush, and not met with in the native huts. Two questions therefore arise—

1. What is the nature of the sporozoites in culex?
2. What is the nature of the sporozoites in anopheles not found in human dwellings?

The sporozoites of culex differed morphologically from those found in the anopheles in the huts, and their arrangement in the salivary glands was different. In the anopheles the sporozoites were present in large numbers. They were in the form of slightly bent sickle-shaped bodies. Those extended by pressure from the glands floated free in the saline solution, and did not remain in the globules of secretion squeezed out of the gland. Stained with hæmatin they showed a very definite outline and a central collection of chromatin arranged in the form of a ring. The sporozoites in the glands of the culex were usually present in fewer numbers in the glands. They were more slender, and more twisted and irregular in outline. When extruded from the gland they for the most part remained included in the mass of secretion, and were less easily seen than the sporozoites in anopheles.

On staining with hæmatin they showed a uniformly staining substance, but no chromatin. They appear therefore to differ from human sporozoites. The possibility of their being proteosoma sporozoites was then considered. Birds of the district were examined, and though halteridium was present in nearly 50 per cent., proteosoma was not encountered. The birds examined included merops, turdus, fringilla, columba, turtur, francolinus, perdrix, psittacus, agapornis, alcedo, laniarius, falco, rallus, grus, musophaga, rhamphastus.

Specimens of the culex were fed upon birds strongly infected with halteridium, but no zygotes were found in six days after feeding. It seems possible these may be the sporozoites of halteridium. We have at present been unable to settle the question, as the supply of this culex suddenly and unexpectedly ceased. Nor at present can we offer any opinion as to the nature of the sporozoites in the large species of

anopheles. Only four of this variety were found by us. It is improbable that they are derived from parasites in monkeys or bats, as the former did not occur in the immediate neighbourhood, and bats, which we were unable to capture, though present, were apparently very few in number.

The sporozoites did not seem to differ essentially from those found in the ordinary anopheles in the huts, but it is certainly difficult on the view that they are human to account for their existence in anopheles at a distance from habitations and in a species not frequenting houses. We are reluctantly compelled to leave these questions unsettled till the onset of the rainy season, when the supply will no doubt be sufficient.

We may thus sum up our conclusions :—

1. Anopheles infest European houses with their adjoining native quarters in the bush.

2. In such houses infected anopheles occur for considerable periods of time, and many of them may be regarded as "fever houses."

3. Native huts and native villages especially are infested with anopheles, and a proportion of these are frequently infected.

4. That breeding-grounds are frequently absent at a time when anopheles swarm in the houses.

5. That swamps form one of the breeding-grounds of anopheles, these collecting in myriads where dwellings are close at hand.

Prophylaxis.

1. The source of greatest danger to Europeans living in the bush is, as we have seen, the presence of huts in which native servants sleep. It is obvious that to have within a stone's throw of a European house hovels in which anopheles thrive and swarm is a most dangerous condition.

In any permanent settlement in the bush it is, then, essential that this source of infected anopheles be removed. The native sleeping-quarters should be far from the European dwellings, in an independent clearing, and natives should not be allowed to sleep under the same roof, or in outhouses, as now is frequently done.

We would also call attention to the danger of sleeping in native huts, or of having one's camp close to a native village, two conditions which are frequently put into practice by travellers in remote districts.

2. Personal precautions, now so usually flagrantly neglected, can be systematically carried out.

We have ourselves never been bitten on the legs or ankles after dark from the simple precaution of changing one's light clothes worn in the day. We now put on boots and gaiters, puttees or mosquito boots, and thick trousers or cord breeches.

We also took minute precautions in the adjustment of our mosquito

nets. These should hang *inside* the poles, and should be turned in under the mattress. The shotted net which is allowed to trail on the ground is objectionable, as during the day mosquitoes lurk under the mattress from which they sally forth at night, being now inside the net. If the net be turned in under the mattress this is impossible. The bell net in use in places is especially ineffective.

On no account should any hole exist in the net, as it is extraordinary to see the pertinacity with which a mosquito will endeavour to find an entry, always successfully if a hole exists.

In the nets we inspected at Mabang all these conditions were violated; it is not surprising then that here quite often we caught fed anopheles inside the nets in the morning.

The inside of the net should be carefully searched with a candle before going to sleep.

Following out these precautions minutely we were able to protect ourselves in the thickly infected compound we have described.

On occasions, however, one is bitten through the net. This is a frequent manner in which men are bitten, consequently we think an important improvement in mosquito nets would consist in a piece of stout cloth or canvas let in at a suitable level so as to protect the limbs during sleep if, as is frequently the case, they come to rest accidentally against the net.

We may refer here to the conditions which we observed in the house boats and river steamers on the Zambesi. It is very usual for new-comers to contract fever on the way up river and so shortly after reaching Blantyre to have a severe attack of malaria. Infection no doubt occurs in the boats or riverside stations. The nets provided not only usually contain holes, but from their small size, necessitated by the cabin, it is impossible that one should fail to be freely bitten through one's limbs and body coming in contact with the net. By putting up each night a full-sized net on the deck of the steamer we escaped being bitten whilst traversing the river.

Everybody ought to possess a large rectangular mosquito net of their own, which they should never on any pretext travel without. In this way one may with care pass through districts rich in mosquitoes and rarely be subjected to their bites.

3. In the next report we shall deal with measures for the destruction of adult anopheles and with the use of repellent bodies, &c.; but in the circumstances described by us we assign to the strictest personal precautions the position of first importance.

Addendum.

a. Description of anopheles found at Mabang in both huts and in remote swamps:—

Total length, 7 mm.

Palpæ, as long as proboscis. Distal end of ultimate segment, white. Junction of ultimate and penultimate also of penultimate and antepenultimate, white. Thorax and abdomen, dark brown.

Legs brown, evenly coloured, except slightly lighter bands at joints and terminal segment.

Wings with four linear dark brown spots on anterior margin.

Smaller specimens were encountered 5 mm. in length; these possessed identical markings with the larger species.

b. Description of culex found at Mabang with sporozoites:—

Total length, 7½ mm.

Palpæ much shorter than proboscis; uniformly light brown, with extreme tip lighter in colour.

Proboscis light brown, with a band, 1 mm. wide, of dark brown near tip.

Thorax and abdomen hairy, yellowish-brown with lighter spots at side of abdominal segments.

Legs, femur, and titræ of all legs mottled light and dark brown. Tarsi markedly banded light and dark brown.

Wings hairy and dull, light brown; no spots.

A Case of Blackwater Fever (*Case VIII*), *with Observations on the History of such Cases.*

F., an engineer on the Sierra Leone Government Railway at Songo Town. Has been in Africa eight years.

His record of recent malarial attacks, kept by the medical officer of the railway, is the following:—

13—18.6.99; 19—22.6.99; 7—10.7.99; 2—5.10.99; 22—25.12.99; 19.1.00, blackwater.

19.1.00. Feeling unwell.

20.1.00. Took phenacetin and quinine, 1 gramme between 12 noon and 1 P.M. Between 4 P.M. and 5 P.M. passed blackwater.

21.1.00. Admitted into hospital in Freetown about midday.

Condition.—Much jaundice, pain in region of gall bladder. Temperature on admission, 102·6°; it then fell to normal gradually, and rose again to 103° before death, on the 23rd.

Patient much troubled by flatulence, thirst, and vomiting. Stupor gradually came on, and patient died 7 P.M., 23rd.

Urine.—Spectroscopically methæmoglobin and much general absorption. Much sediment, consisting of spherical granules of varying size, from that of a red corpuscle to half or less. Sediment stained yellow, identical in appearance with that described in Case I, and resembling in nature granular tube casts, indicating destructive kidney changes.

On the 23rd a few ounces only passed of the same character.

On the 22nd and 23rd the action of the urine was tried upon—

1. Normal blood,
2. The patient's blood.

The observations were made on the stage of the hæmocytometer, and corpuscles counted at intervals of an hour. In neither case was any hæmolysis apparent.

Blood.—Examinations were made on the 21st, 22nd, and 23rd. No parasites were found. A careful examination of the leucocytes was made. The leucocytic variation that we have described in previous cases was not present. We have already alluded to the fact that we have observed its absence in ordinary malaria. The leucocytic values were—

21.1.00.	Large mononuclear	8	per cent.	500 counted.
	Small „	8	„	„
	Polynuclear	84	„	„
22.1.00.	Large mononuclear	11	„	„
	Small „	4	„	„
	Polynuclear	84	„	„
23.1.00.	Large mononuclear	12	„	„
	Small „	7	„	„
	Polynuclear	80	„	„

So that in spite of the large polynuclear excess, the large mononuclear value is as high or even somewhat higher than that of the lymphocytes. A parallel condition occurred in Case II, where, coincident with fatal symptoms setting in, there was a sudden inroad of polynuclears; but still with the large mononuclears in excess of the small mononuclears.

Pigment was looked for, and one typical large mononuclear pigmented leucocyte with pigment clumps was found on the 21st. On the 22nd it was thought that in many leucocytes, mononuclear and polynuclear, extremely fine granules of pigment could be seen. The extremely fine character of the pigment here, and the almost total absence of clumps, is interesting when we compare this condition with that found in the spleen post-mortem.

Tonicity of the Blood.—Observations were made on the 22nd, and we may here recall the fact that there was still hæmoglobinuria on the 23rd, though on both the 22nd and 23rd no hæmoglobinæmia was to be detected by the spectroscope.

A series of salt solutions with a difference of 0·01 gramme per cent. between each member of the series was used. A loopful of blood was added to each of these tubes, containing $\frac{1}{2}$ c.c. of each strength. The corpuscles were allowed to settle, and the result was then recorded—

	Salt solution 1.	Salt solution 2.	Salt solution 3.	Salt solution 4.	Salt solution 5.
Patient	No H	No H	No H	No H	No H
Control 1	No H	No H	*h*	*h*	H
Control 2	*h*	*h*	H	H	H

Note.—H means hæmolysis, *h* = slight hæmolysis.

It is thus seen that there is a striking difference in the tonicity of the blood of this patient and that of control, (1) whose blood may be considered normal, never having suffered from malaria and control; (2) who had suffered from severe attacks of malaria six months previously.

The remaining corpuscles of the patient, then, have an increased resistance. This would appear to be brought about by the destruction of the low-resistance groups of corpuscles, leaving only those of higher resistance. The clinical importance of the observation lies in the possibility of determining whether a patient has previous to his attack a number of corpuscles of high tonicity, *i.e.*, low resistance. We append at the end of this report a few observations made on subjects suffering more or less constantly from malaria, in whom a distinctly raised tonicity could be shown to exist.

It is probable that such a condition is one favourable to the action of a hæmolytic agent. If this view be correct, two factors will determine the immediate recurrence of relapses:

(1) The hæmolytic agent may be sufficiently strong to dissolve still more of the residue of cells of low tonicity.

(2) On the character of the regenerated cells. If the tonicity of the now increased total still remains low, the condition will be unfavourable to further hæmolysis; if, on the contrary, the tonicity is raised, or comes back to normal even, then a recurrence is more probable.

Blood Serum.

22.1.00. Serum markedly yellow in colour. The colouring matter was in such quantity that two small loops of blood coloured 1 c.c. of saline distinctly.

23.1.00. The serum was allowed to separate from the clot in a pipette. Deep yellow in colour. Neither in this or the preceding specimen was there any hæmoglobin in the serum. There were no urobilin bands, but only general absorption of the blue end of the spectrum. The colouring matter was probably bilirubin; but we were unable at the time to apply further tests

The serum separated from the clot had no hæmolytic action on normal blood cells. Nor did the serum become tinged on being left in contact with the patient's blood cells.

In Case III we have already noticed this deep yellow serum and the absence of hæmoglobin in the serum at the time of examination.

Post-mortem. 13½ *hours after death.*

Marked yellow colour of conjunctivæ and skin. Subcutaneous tissues, omentum, and peritoneum deeply stained.

Liver.—Enlarged. 72 ozs. Soft and flabby. Surface shows dark brown mottling. On section, brownish-yellow.

Spleen.—Much enlarged. 26 oz. Soft and diffluent.

Kidney.—Capsule slightly adherent in parts. On section yellowish staining evident. Cortic has areas of congestion.

Brain.—Slight œdœma over convolutions. No pigmentation apparent.

Bone Marrow.—Red marrow of sternum can be squeezed out in large amount as a thick substance like anchovy sauce.

Bile.—The gall bladder contained a quantity of very thick, almost solid black tarry bile.

Pancreas.—Normal in appearance.

Smear preparations and sections were made of spleen, liver, kidney, brain, and bone marrow.

Spleen.—A large amount of melanin was present, chiefly in large macrophages and in mononuclear elements, but also to a less extent in the cells of the splenic reticulum. The pigment occurred in masses and in typical clumps. Cells containing fine granules were very frequent.

Yellow pigment was present, chiefly in the macrophages. No parasites were found.

Liver.—The liver cells contained large numbers of coarse granules of golden yellow pigment. Many large swollen endothelial cells were present in the capillaries, and contained clumps and isolated grains of melanin. Grains of melanin were also present in many normal endothelium cells.

A certain amount of yellow pigment was also present in the large endothelial cells.

In both the liver and spleen small yellow acicular crystals were present in large numbers. Many of these were present in endothelial cells, and were arranged around the nucleus. They were insoluble in dilute acids. They did not give any Prussian-blue reaction with ferrocyanide of K and hydrochloric acid. They were, however, dissolved on prolonged treatment with xylol.

No parasites were found.

Kidney.—The glomeruli appeared normal. No parasites were to be seen in the blood corpuscles in the glomerular capillaries.

The epithelium of the convoluted tubules was granular, and to a large extent shed into the lumen. The staining reactions of the cells were also impaired.

The straight and collecting tubules were all closely packed with granular material of a yellowish colour. The granules varied from the size of a red corpuscle to one-fifth or less. The granular substance was similar to that present in the urine during life, and to that described in Case I of our previous report. They consisted of altered epithelial cells.

The kidney vessels were engorged. There was no leucocytic infiltration. No parasites were found.

Bone Marrow.—A large amount of both yellow and brown pigment was present. Many of the mononuclear elements contained numerous coarse granules of yellow pigment resembling those in the liver cells. Others contained clumps and fine granules of melanin.

Nucleated blood cells were present. Neither the multinucleated cells nor the cells with branched nuclei contained pigment. No parasites were found.

Brain.—No parasites were found.

Bile.—The bile contained numerous irregular yellow nail-shaped crystals. These were unchanged by strong HNO_3. They did not give the Prussian-blue reaction with ferrocyanide of K and HCl. They were soluble in strong alkali.

This preliminary examination of the organs is sufficient, then, to establish the diagnosis of malaria.

We have, then, found malarial pigment in abundance in all our five post-mortem cases.

History of Blackwater Cases.

We have already alluded to the difficulty of obtaining accurate histories, and have asserted our belief that statements of patients unsubmitted to rigorous cross-examination are valueless. We give two instances of this, together with a summary of the histories of some cases of blackwater that have recently been published :—

A. Trader, Sherboro, Sierra Leone. Had fever during his first six months in the country.

8.1.00. Feeling ill in the afternoon, went to bed. 10 P.M. took quinine, 0·6 gramme. 11.30 P.M. a West African medical man gave him more quinine.

9.1.00. 2 A.M., blackwater.

15.1.00. First seen. No parasites or pigment; urine normal. Invalided to England.

This case is instructive, because the patient when first asked said he had not taken any quinine (this meant, as afterwards appeared, that he did not take 5 grains daily).

The case was subsequently quoted as one where the action of quinine could be excluded, although a careful examination elicited the above facts, and that "he, of course, took quinine when feeling out of sorts."

B. Case VIII. Railway accountant, Songo Town, Sierra Leone. According to the patient's statement he had lately enjoyed good health, but the record kept by the medical officer to the railway showed the exact reverse to be the case. The patient probably meant that he had not been suffering from severe fever with high temperatures (105°), as it is quite common to ignore slight but quite definite attacks of malaria.

As this was the fifth case of blackwater that had occurred among the residents at Songo Town within the space of about four months, we determined to examine the conditions under which the railway officials lived there. These are fully described in Part II of our report on the distribution of anopheles in Sierra Leone. Here it will suffice to say that malarial fever was constantly occurring in the small area; that at the time of our visit anopheles with sporozoites were found in this group of houses, so that the presumption was very great that cases of blackwater arising here had a malarial origin.

In Case VIII of our series, as in the majority of our cases, no parasites were found. Had not the post-mortem given clear evidence of malaria we should during life, had the patient recovered, have classed it doubtfully as a case of malaria; but when we consider the case further in the light of the previous history of the patient, the data would we believe have been sufficient to make it certain that the case was malarial, apart from other evidence.

We think it then of importance that the histories of similar cases should be minutely inquired into, and mere casual statements not be accepted as evidence.

It is instructive, then, to consider what have been the histories of these five cases of blackwater, only the last of which we saw.

The data are incomplete, but knowing that this little colony of five persons lived in the midst of infected anopheles, and that actual records exist of the fevers of some of them, we have little doubt that the blackwater fever was in these cases the last of a series of malarial attacks. The patients had malarial fever on the following dates :—

B. 13th—18th, and the 19th—22nd of June, 1899. 7th—10th July, 1899. 2nd—5th October, 1899. 22nd—25th December, 1899. 19th January, 1900. Blackwater.

	July.				August.					September.				October.			
Weeks	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Songo—																	
House 1	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..
" 2	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..
" 3	1	2, B	..	1	2	..	..	..	..	..	..	..	..	..	..	..	..
" 4, 5, and 6	..	1	3	..	..	1	..	..	..	..	2	1	1	2	2	2	..
Mabang—																	
House 7 and 8†	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	1
" 9	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..
Camp 1	..	..	1	1	2	1	..	1	..	..	1	..	1	..	..	..	..
" 2	..	..	..	1	..	..	..	..	..	..	..	..	..	..	1	..	1

	November.					December.				January.				February.			
Weeks	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Songo—																	
House 1	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..
" 2	..	..	B*	..	..	..	1	..	..	..	..	..	..	..	1	..	1
" 3	..	1	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..
" 4, 5, and 6	..	2	..	1	..	..	..	2	1	..	..	B	..	..	..	..	..
Mabang—																	
House 7 and 8	..	..	..	..	..	..	..	..	2	1	1	3	2	..	1	..	..
" 9	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..
Camp 1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
" 2	2	..	..	..	..	1	1	..	..	..	..	..	..	..	..	..	..

B signifies a case of blackwater.

* Had previously been on many expeditions, &c., and had suffered from malaria before coming to house 2.

† Houses occupied from second week in October.

C. 24th—30th May, 1899. 6th—10th June, 1899. 27th—30th July, 1899. 6th August, 1899. Blackwater.

D. Previously in India. Only out three weeks. Ill more than half the time. Blackwater.

E. Blackwater on voyage home.

F. Much fever. Blackwater. 24.11.99.

We thus see that in this group of houses the officials are frequently suffering from malarial fever, and they have furnished five cases of blackwater.

A detailed account of the malarial histories of these houses is given in the table, together with three of the cases of blackwater.

We may add two cases that came under our observation in British Central Africa. In the house where Case I of our series died there occurred, about four months after, a very severe case of malaria. Similarly the house in which Case III lived was occupied, on his removal to hospital, by his brother, who ten days after his arrival was admitted into hospital with typical malaria.

Besides the previous history of these cases the subsequent history of the patient not infrequently throws light on the nature of the blackwater process. This is especially valuable where parasites are absent, as has been the case in all our cases with the exception of Case II, where parasites existed in large numbers before the attack of blackwater. We have already shown in Case IV how our diagnosis of malaria, though parasites were absent, was established by a relapse of malaria; in Case V how blackwater occurred in the interval between two malarial attacks. We consequently think that if the histories of these cases were only accurately kept, much evidence might be accumulated. It is only rarely that here in West Africa as in Central Africa a microscopical examination has been made, whereas one might almost say in malarial countries the microscope should replace the stethoscope.

In the absence of microscopical evidence we may yet hope for help by a careful record of the histories of cases. We may illustrate this by a review of Koch's 'Ueber Schwarzwasserfieber' and Ollwig's 'Ein Beitrag zur Behandlung der Malaria mit Methylenblau.'

In Koch's publication seventeen cases are recorded. These may be divided thus:—

1—7. Negative. Including two cases where no pigment was found (post mortem) in sections.

8. Positive. Ring forms, unpigmented.

9. Positive. Double tertian.

10. Positive. Many relapses in Germany. Diagnosis, tertian.

11. Positive. Relapse in Berlin. Diagnosis, double tertian.

12. Positive. Numerous ring parasites.

13. Positive. Relapse nine days after attack of blackwater.
14. Positive. Large pigmented tertian parasites some days before the attack.
15. Positive. Relapse in a fortnight.
16. Positive. Numerous ring-form parasites some days before attack.
17. Positive. Parasites before and after attack.

Thus out of seventeen cases we have evidence of malarial infection in ten. Of these, four were relapses.

More striking still are the six cases recorded by Ollwig, in all of which there was evidence of malaria. Three of these are included in Koch's series, leaving three cases of Ollwig.* A brief note of his cases is appended.

I. 16.7.98. Blackwater in Germany. No parasites.

31.7.98. Relapse of malaria. Parasites, small ring forms. Diagnosis, mal. tropica.

II. "He quite lately observed that his urine became dark and slightly reddish after taking only 0·5 gramme of quinine, which dose he was then using."

13.9.98. Parasites. Diagnosis, tertiana duplex and malaria tropica.

III. Two and a half years in German East Africa; much fever there and on voyage home. In Germany.

June, 1898. Blackwater. No parasites.

30.7.98. Parasites. Diagnosis, mal. tropica.

13.8.98. Parasites. Diagnosis, mal. tropica.

Ollwig's remaining three cases are identical with Cases 10, 11, 17 in Koch's series.

The subsequent history of these cases then clearly shows the existence of the malarial infection, which at the time of the blackwater attack was not evident. If microscopical examinations were constantly made in malaria we should have before and immediately preceding the blackwater attacks evidence, we feel certain, of their malarial origin. The value of the later histories of the patients and of microscopical examinations, Ollwig's cases clearly show.

It may be said that some of these cases are fresh infections, but a consideration of the conditions in British Central Africa and West Africa make it only too evident that blackwater patients have long been suffering from malarial fever which eventually culminates in blackwater.

* Ollwig, "Zeitschrift für Hygiene und Infectionskrankheiten," vol. 31, p. 317.

Tabular Arrangement of Twenty-eight Cases of Blackwater.

Author.	No.	Negative or positive.	Evidence for malarial infection.	Quinine history. Intervals in hours.
Koch	1	No	.. ..	Yes, 3 hrs.
	2	No	.. ..	No history
	3	No	.. ..	Yes, some hours
	4	No	.. ..	Yes, a few hours
	5	No	No pigment in spleen	Yes, one hour
	6	No	No pigment in liver or spleen	Yes, a short time
	7	No	.. ..	Yes, $1\frac{1}{2}$ hrs.
	8	Yes	Ring from parasites unpigmented	Yes, 3 hrs.
	9	Yes	Double tertian parasites	Yes, a few hours
	10	Yes	Many relapses in Germany. Tertian	Yes, soon after
	11	Yes	Relapses in Berlin. Double tertian	Yes
	12	Yes	Numerous ring parasites	{ No; Yes, 8 hrs.; Yes, 8 hrs. }
	13	Yes	Relapse 9 days later	Yes, some hours on three separate occasions
	14	Yes	Large pigmented tertian parasites some days before attack	Yes, very soon after
	15	Yes	Relapse in a fortnight	Yes, some hours
	16	Yes	Numerous ring parasites some days before attack	{ No; Yes, 3 hrs. }
	17	Yes	Parasites before and after attack	{ Yes, $2\frac{1}{2}$ hrs.; Yes, 2 hrs.; Yes, $2\frac{3}{4}$ hrs. }
Ollwig	18	Yes	B.W. in Germany. Relapse in a fortnight Parasites, malaria tropica	Yes, some hours
	19	Yes	In Germany. Parasites, malaria tropica, malaria tertiana duplex	Yes
	20	Yes	B.W. in Germany. A fortnight later parasites, malaria tropica	No history
Malaria Commission	21	Yes	No parasites. Mononuclear variation, much pigment during life, also post-mortem	Yes
	22	Yes	Parasites numerous before attack, no parasites after. Much pigment and developing crescents post-mortem	[Yes] ?, 23 hrs.
	23	Yes	No intracorpuscular parasites. Mononuclear variation pigment, a very few crescents, relapse of malaria tropica	Yes { 10 hrs.; 4 hrs. }
	24	Yes	No parasites. Mononuclear variation, pigment scanty	Yes, $7\frac{1}{2}$ hrs.
	25	Yes	B.W. between two attacks of malaria tropica	Yes
	26	Yes	Much pigment in spleen	No history
	27	Yes	Much pigment in spleen	No history
	28	Yes	No parasites. No mononuclear variation pigment during life, much pigment in spleen, liver, and bone-marrow	Yes, 4 hrs.

Analysis of Twenty-one Post-mortem Examinations of Cases of Blackwater Fever.

Author.	No.	Negative or positive.	Evidence for malarial infection.
Koch	1—2	Negative	No parasites, no pigment.
Thin*	3	Yes	Pigment very scanty in spleen, sporulating parasites in brain.
F. Plehn†	4—16	Yes	In all malarial pigment in organs, but sometimes very little.
Malaria Commission	17—21	Yes	In all malarial pigment in abundance.

In 75 per cent. of these cases and 90 per cent. of the post-mortems, then, there is satisfactory evidence of malarial infection. That a large proportion of cases of blackwater should, however, be described as non-malarial is not surprising. In all our cases, although malaria was undoubtedly present, yet in none of the cases were parasites present during or immediately after the attacks.

This absence of parasites indeed is the most frequent condition in blackwater, and is a strong argument against the special parasite theory. Thus, in Texas fever Koch notices that the number of parasites seen in the blood is proportional to the extent of hæmoglobinuria, the exact reverse of blackwater.

That when parasites are found they are aestivo-autumnal or tertian forms indistinguishable from the ordinary types, is also against the view of a special parasite.

Still another strong argument is the fact that infection so rarely takes place before the patient has been a considerable time in the country, one or two years; whereas malaria in man and Texas fever in cattle affect man and animals respectively independently of the time of residence.

That blackwater should not occur for a considerable time after a patient's arrival is indeed in accordance with the view that it is a condition induced by frequent attacks of malaria, which after a time under certain conditions may give rise to blackwater.

In favour of this view, too, is the fact that in the cases of blackwater on the Sierra Leone railway the patients were living in houses where anopheles were found infected with sporozoites, and that the previous histories were undoubtedly malarial.

Koch recently and F. and A. Plehn previously have confirmed the early observations of Tomaselli that quinine is a cause, and indeed the

* 'Lancet,' 1899.

† Private communication to Malaria Commission.

most frequent cause, of hæmoglobinuria. We have not seen any case in which quinine could be excluded, and in most of our cases the interval between the administration of quinine and the onset of hæmoglobinuria was very constant, *i.e.*, from four to ten hours. We have ourselves had one case in hospital in which quinine appeared definitely to induce a severe attack of hæmoglobinuria. What the conditions are which determine whether quinine or other causes in a malarial patient can induce blackwater remain still to be established.

Observations on the Tonicity of the Blood of Persons suffering periodically from Malarial Attacks.

	1st salt solution.	2nd.	3rd.	4th.	5th
1. Control	trace	H	H	H	H incomplete
2. L. .... 6 months in Africa.	trace	H	H	H	H incomplete
3. M.	*h*	CH	H much	CH	CH
4. H.	*h*	CH	H slight	CH	CH
5. B.	*h*	CH	H much	CH	CH

h = slight hæmolysis, H = considerable, CH = complete.

A second observation was made a few days later on M, with a different control in this case. The result was :—

	Salt 1.	2.	3.	4.	5.	6.	7.
1. Control ..	No H	*h* faint tinge	*h* faint tinge	*h*	H	H incomplete	H incomplete
2. M.	*h* strong tinge	*h* strong tinge	*h* strong tinge	CH	CH nearly	CH rapidly	CH rapidly

The result then agrees very closely with the first observation. It is difficult without making the test a quantitative one, as by using colorimetrical methods, to express exactly differences in words, but to the eye the differences are clearly visible. The blood of M, also of H and B, showed then a raised tonicity, whereas that of L, who had been in West Africa only six months, was equal to that of the control.

It must be noted further that unfortunately different series are not comparable one with the other owing to the fact that on each occasion different salt solutions had to be employed.

Addenda.

I.

After this report had been written we had the opportunity of examining a patient convalescent, from blackwater, ten days. His tonicity was again found to be lowered when compared with a normal control, thus :—

	1st strength of salt.	2nd.	3rd.	4th.	5th.
Patient	No H	No H	H	H	H
Control	No H	H	H	CH	CH

The difference in strength between each salt solution was 0·01 gramme per cent. ; in the 3rd, 4th, and 5th dilutions the amount of hæmolysis was in each case less than in the corresponding normal control. (CH = complete hæmolysis.)

As the result then of these few preliminary observations it appears that persons suffering periodically from malaria have a raised tonicity, while in these two cases of blackwater the tonicity was lowered—in the fatal case markedly so, in the convalescent less markedly.

II.

The condition of the blood in the convalescent from blackwater is an interesting one. We have given above the history of the case. The patient was first seen on the sixth day after the attack. An examination of the leucocytes made it evident simply by inspection that there was a mononuclear increase.

A count of 1200 leucocytes gave the following values :—

Large mononuclear	22·7
Small mononuclear	12·6
Polynuclear..................	64·7

and although at first we considered that pigment was absent, yet on a more prolonged examination several leucocytes, chiefly mononuclear, contained fine granules.

We have already alluded to the persistence of this mononuclear variation in malaria after subsidence of all symptoms and disappearance of parasites from the circulation. The following two cases further illustrate this point:—

Case I. 27.2.00. Blood. Numerous parasites. Quinine gr. x were then taken.

28.2.00. Quinine gr. xv.

1.3.00. Quinine gr. xxv.

3.3.00. Blood examined. No parasites. Pigment extremely scanty, one typical large mononuclear leucocyte only being found. Leucocytic count (500).

Large mononuclear	24
Small mononuclear	24
Polynuclear	52

Case II. 24.2.00. Severe attack of malarial fever; then quinine taken daily.

1.3.00. Pigmented leucocytes found, three in number. Leucocytic count (500).

Large mononuclear	29·6
Small mononuclear	18·8
Polynuclear..................	51·6

Accordingly the conditions of the blood of this patient convalescent from blackwater and of the malaria patients exhibit a close parallelism.

"A New Anopheles (*A. paludis*) from Sierra Leone." By F. V. Theobald, M.A. Communicated by E. Ray Lankester, M.A., F.R.S. Received May 9, 1900.

Anopheles paludis, n.sp.

♀ Head dark brown with a few curved white scales in front, upright white ones in the middle and upright black ones at the sides, a tuft of yellowish hairs projecting forwards; antennæ dark brown to almost black, basal joint dark, the whole covered with a white pubescence, verticils dark brown, the first three or four basal joints with a few white scales; palpi densely covered with black scales with four narrow rings of white scales; proboscis covered with black scales, testaceous at the tip.

Thorax brown with a greyish tomentum longitudinally adorned with darker lines and with scattered yellowish, curved, hair-like scales;

scutellum brown with a purplish tinge, and edged with chestnut-brown bristles; metanotum deep brown; pleuræ dark brown with a few pale reflections.

Abdomen deep steely black, with a few small irregular deep ochraceous marks seen in some lights, covered with deep-brown hairs, which appear black in some lights.

Legs deep ochraceous, with dark-brown scales, the apices of the metatarsus and first tarsal joint of the fore and mid legs with a narrow yellowish apical band; hind legs with the metatarsus very long and thin, the extreme tip of the first tarsal joint and the whole of the three following joints pure white like *A. dubius*, mihi; in the fore and mid legs the last tarsal joints are slightly paler than the rest of the legs, of a somewhat ochraceous tinge in certain lights; ungues dark brown, untoothed.

Wings very much as in *Anopheles sinensis*, Wied., veins clothed with creamy yellow and black scales, the costa dark, broken by two small yellow spots, one near the apex and the other about a third of the length from the apex, the apical spot extending on to the first long vein and the upper fork of the second long vein, the other spot passes into the first long vein only; there is also a yellow spot nearer the base on the same vein, which, however, does not reach the costa; fringe of the wings dark, except where the lower branch of the fifth long vein joins the border, where the cilia form a broad yellow spot.

Length.—5 to 5·5 mm.

Habitat.—Katunga, Sierra Leone.

Date of Appearance.—January.

Observations.—Three specimens of this species received from Mr. S. R. Christophers, only one at all perfect as far as the legs go, but luckily all parts were present on combining the three, so a complete description can be given.

With the hind legs absent this species closely resembles *A. sinensis*, especially in regard to the wings; the white tarsi in the hind legs, however, clearly separate it from that species. The white tarsi make it resemble *A. dubius*, but the wings differ; in this species the fringe has only one pale patch at the end of the lower half of the fifth vein, whereas in *A. dubius*, mihi, there are several small pale patches; there are also only two pale costal spots as in *A. sinensis*.

Mr. S. R. Christophers, who sends this species, says in his letter that "it occurs very infrequently, and we have been unable to obtain any males. It occurs widely distributed, but rarely, in the Sierra Leone swamps. This species has been shown on two occasions to contain sporozoites in the salivary glands, though caught about a quarter of a mile from any human habitation."

It is undoubtedly a distinct species, coming near *A. dubius*.

scu
bris
refl
A
ma
bla

me
ye
th

s